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Human antithrombin III mutants.

A novel human antithrombin III (AT III) mutant having a high antithrombin activity in the absence of heparin deffective in the treatment of thrombotic disorders as an anticoagulant, which is obtained by mutating amino ds at the reactive site and the heparin binding site of human AT III into another amino acids with the use of a DNA coding for AT III as a template.

A method for mass producing the above-described mutant by incubating a host transformed by an pression vector having the cDNA of the mutant inserted therein.

Background of the Invention

Field of the Invention

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The present invention relates to a human antithrombin III (AT III) mutants which are obtained by mutating one or more amino acid(s) in the amino acid sequence of human AT III into another amino acid(s) and exhibit high antiprotease activities even in the absence of heparin. These human AT III mutants are usable as a remedy for thrombotic disorders.

Description of the Related Art

Anticoagulant activity of glycosaminoglycans including heparin is mediated by antithrombin III (AT III) and heparin cofactor II (HC II) contained in the blood. AT III and HC II are serine protease inhibitors which are called serpins in general. There has been often reported with respect to AT III among these substances that a decrease in the blood AT III level due to a congenital or acquired factor would result in thrombotic disorders. Accordingly, AT III plays a physiologically important role as a factor regulating the blood coagulation system consisting of a series of serine proteases.

It is known that human AT III is a glycoprotein of a molecular weight of approximately 60 kd which is mainly synthesized in the liver and contained in normal plasma at a concentration of about 150 µg/ml and that human AT III inhibits serine proteases participating in coagulation and fibrinolysis systems including thrombin and factor Xa. The primary structure of human AT III has been clarified by the direct determination of its amino acid sequence (see Petersen, T.E. et al., The Physiological Inhibitors of Blood Coagulation and Fibrinolysis, Elsevier Science Publishers, Amsterdam, 43, 1979) and cDNA cloning [see Bock, S.C. et al., Nucl. Acids Res., 10, 8113 (1982); Prochownik, E.V. et al., J. Biol. Chem., 258, 8389 (1982); Chandra, T. et al., Proc. Natl. Acad. Sci. USA, 80, 1845 (1983)]. According to these reports, human AT III is a single-chain glycoprotein consisting of 432 amino acids which is secreted and formed by excising a signal peptide of 32 residues from a precursor protein. It has four N-linked glycosylation sites in the molecule. The carbohydrate content is about 15% of the molecular weight.

Human AT III reacts with a serine protease such as thrombin at a ratio of 1:1 and thus forms a stable complex, thus inhibiting the activity of the protease. It is thought that, in this reaction, a peptide bond between the 393rd Arg residue and the 394th Ser residue in the molecule of human AT III is cleaved by the protease and an acyl bond is formed between the terminal Arg residue newly formed and the Ser residue at the active center of the protease. This Arg (393)-Ser (394) sequence is generally referred to as a reactive site.

The protease inhibition by AT III would relatively slowly proceed. When the reaction system contains heparin, however, the reaction is dramatically accelerated. Namely, the addition of heparin elevates the thrombin inhibition rate of AT III by more than 1,000 times. It is thought that this function mechanism proceeds as follows. When heparin binds to a specified site (heparin binding site) in AT III, the higher-order structure of AT III turns into a structure liable to undergo interaction with the protease. At the same time, the protease binds to the heparin molecule. Thus a ternary complex is apt to be formed. Further, from the physiological viewpoint, it is considered that heparin-like substances existing on the surface of vascular endothelial cells exert similar actions and thus play an important role in the mechanism for regulating the blood coagulation system by AT III.

There have been used so-called anticoagulants for treating and preventing thrombotic disorders induced by various causes. Heparin is one of highly important anticoagulants at present. However, it is reported that serious side effects are sometimes induced by the administration of heparin [see Amerena, J. et al., Adverse Drug React. Acute Poisoning Rev., 9, 1 (1990); Levine, M.N. et al., Semi. in Thrombos. Hemostas., 12, 39 (1986); Kelton, J.G. et al., ibid., 12, 59 (1986); Levine, M.N., ibid., 12, 63 (1986)]. Typical examples of these side effects include hemorrhage, thrombocytopenia, hypoadrenalism, hypersensitiveness, necrosis of the administration site and osteoporosis. When there is a high risk of hemorrhage in the fields of, for example, obstetrics and gynecology or postoperative treatments or in the case of a prolonged administration, heparin should be carefully used. Furthermore, it is reported that heparin promotes inactivation of AT III by elastase of neutrophils in vitro [see Jordan, R.E. et al, Science, 237, 777 (1987); Jordan, R.E. et al., J. Biol. Chem., 264, 10493 (1989)]. Thus care should be taken in the administration of heparin when elastase of neutrophils seemingly relates to the conditions of diseases such as serious infection or septicemia. In addition, the anticoagulant effect of heparin is essentially mediated by AT III and, therefore, can be scarcely expected in the case where blood AT III level is lowered.

Meanwhile, human AT III has been clinically applied to thrombophilia based on congenital AT III deficiency and disseminated intravascular coagulation syndrome (DIC) accompanied by a decrease in AT III in the form of a plasma derived AT III concentrate. As described above, however, AT III exhibits only a slow progressive antithrombin activity in the absence of heparin. Therefore the use of AT III alone is rather a supplementary treatment and its usefulness as an anticoagulant is limited. Thus attempts have been made to use AT III together with heparin or to prepare and use an AT III/heparin complex to thereby improve the usefulness of AT III as an anticoagulant. However, it is obvious that the above-mentioned disadvantages of heparin cannot be overcome even by these methods.

As described above, AT III has two functional sites, namely, the reactive site and the heparin-binding site. A number of reports have revealed that the amino acid sequence around the reactive site carries an important role in the expression of the function as a prolease inhibitor as well as in the determination of inhibition specificity against various proteases. In congenital AT III anomaly such as AT III Hamilton wherein Ala at the 382-position has mutated into Thr [see Devraj-Kizuk, R. et al., Blood, 72, 1518 (1988)], AT III Cambridge I wherein Ala at the 384-position has mutated into Pro [see Perry, P.J. et al., FEBS Lett., 254, 174 (1989)], AT III Glasgow wherein Arg at the 393-position has mutated into His [see Erdjument, H. et al., J. Biol. Chem., 263, 5589 (1988)], AT III Pescara wherein Arg at the 393-position has mutated into Pro [see Lane, D.A. et al., J. Biol. Chem., 264, 10200 (1989)] and AT III Denver wherein Ser at the 394-position has mutated into Leu [see Stephens, A.W. et al., J. Biol. Chem., 262, 1044 (1987)], abnormal AT III molecules each has lost antiprotease activity and patients of these anomalies suffer from thrombotic disorders.

On the other hand, studies on congenital AT III molecule anomaly and results of chemical modification of amino acid residues have revealed amino acids directly relating to the heparin-binding site, namely, binding to heparin. Regarding the molecular anomaly, there have been reported AT III Rouen III wherein IIe at the 7-position has mutated into Asn [see Brennan, S.O. et al., FEBS Lett., 237, 118 (1988)], AT III-Rouen IV wherein Arg at the 24-position has mutated into Cys [see Borg, J.Y. et al., FEBS Lett., 266, 163 (1990)], AT III Basel wherein Pro at the 41-position has mutated into Leu [see Chang, J.Y. and Tran, T.H., J., Biol. Chem., 261, 1174 (1986)], AT III Toyama wherein Arg at the 47-position has mutated into Cys [see Koide, T. et al., Proc. Natl. Acad. Sci. USA, 81, 289 (1984)] and AT III Geneva wherein Arg at the 129-position has mutated into Gln [see Gandrille, S. et al., J. Biol. Chem., 265, 18997 (1990)]. Each of these abnormal AT IIIs has a lowered heparin affinity and cannot exert normal physiological functions, thus causing thrombotic diorders. Further, the results of experiments on chemical modification of amino acids suggest that amino acids including Trp at the 49-position, Lys at the 114-position, Lys at the 125-position, Arg at the 129-position, Lys at the 136-position and Arg at the 145-position might directly relate to binding to heparin [see Blackburn, M.N. et al., J. Biol. Chem., 259, 939 (1984); Peterson, C. et al., J. Biol. Chem., 262, 8061 (1987); Sun, X.J. and Chang, J.Y., Biochemistry, 29, 8957 (1990)].

Based on these findings, attempts have been made to improve AT III through substitution of an amino acid(s) of AT III. For example, ZettlemeissI et al. have disclosed a method for producing an AT III mutant having improved properties relating to heparin binding/heparin activation by mutating an amino acid(s) at the glycosylation site in AT III and another method for producing an AT III mutant having modified enzyme specificities by mutating an amino acid(s) at the reactive site (European Patent Publication-A No. 384122). Further, Dijkema et al. has reported a method for producing an AT III mutant having a modified antithrombin/antiXa activity by mutating an amino acid(s) at the reactive site (International Publication No. WO 91/00291).

However there has not been found out any human AT III mutant which is satisfactory from the clinical viewpoint. It is, therefore, urgently required to construct a human AT III mutant having an elevated activity of inhibiting thrombin or factor Xa in the absence of heparin.

It is an object of the present invention to provide novel human AT III mutants having a high antithrombin activity even in the absence of heparin. It is another object of the present invention to provide a method for mass producing said human AT III mutants by the recombinant DNA technology.

Disclosure of the Invention

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Summary of the Invention

At present, it is thought that the mechanism of enchancing the antiprotease activity of AT III by heparin would proceed as follows. First, heparin binds to the heparin binding site of AT III to thereby change the conformation of AT III into another one which can more easily react with a protease. At the same time, the protease binds to the same heparin molecule at the above-mentioned heparin binding site, thus elevating the rate of the formation of an AT III/protease complex [see Pletcher, C.H. and Nelsestuen, G.L., J. Biol.

Chem., 258, 1086 (1988)]. According to this hypothesis, the change in the configuration at the reactive site induced by the heparin binding to the heparin binding site of AT III is thought to be important in the enhancement of the antiprotease activity. This fact suggests that an AT III mutant exhibiting an enhanced protease activity in the absence of heparin can be constructed by artificially modifying the amino acid sequence in the neighborhood of the reaction site to thereby change the configuration at the reactive site.

If an AT III mutant having an enhanced antithrombin activity in the absence of heparin can be obtained based on the above-mentioned idea, the action of binding to heparin is seemingly not an important characteristic of this AT III mutant. Thus it is conceivable that a reduction in the affinity for heparin caused by introducing an amino acid substitution into the heparin binding site of the above-described AT III mutant would scarcely affect its function, different from the above-mentioned AT III TOYAMA and AT III GENEVA wherein a mutation in the heparin binding site results in abnormalities in the function. It is rather expected that the clinical usefulness of AT III mutant might be enhanced thereby, since interactions with heparin-like substances existing on the surfaces of vascular endothelial cells are suppressed and thus the half-life in the blood is prolonged and the inactivation with neutrophil elastase is avoided.

Based on this idea, the present inventors have conducted extensive studies in order to improve human AT III. As a result, they have successfully constructed the desired novel human AT III mutants, thus completing the present invention.

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Accordingly, the present invention relates to a human antithrombin III (AT III) mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence described in sequence ID No. 2 except that an amino acid(s) mutates into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41- to 47-positions, the 125- to 133-positions and the 384- to 398-positions.

Namely, the present invention relates to an AT III mutant which is a mutated human AT III characterized in that at least one amino acid in each of four regions of the 11- to 14-positions, the 41- to 47-positions, the 125- to 133-positions and the 384- to 398-positions has mutated, either singly or combinedly, into another amino acid(s), or an AT III mutant characterized in that in the amino acid sequence of human AT III, one or more amino acid(s) selected from among those at the 11- to 14-positions, the 41- to 49-positions, the 121- to 135-positions and the 384- to 398-positions have mutated into another amino acid(s) and the antithrombin activity in the absence of heparin is elevated as compared with natural AT III.

The human AT III mutant according to the present invention includes the following embodiments:

- (1) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that an amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41- to 47-positions, the 125- to 133-positions and the 384- to 398-positions mutates into another amino acid(s) selected from the group consisting of Ala, Gly, Trp, Pro, Leu, Val, Phe, Tyr, Ile, Glu, Ser, Gln, Asn and Arg.
- (2) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 384- to 398-positions and that an amino acid(s) mutates into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41- to 47-positions and the 125- to 133-positions.
- (3) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 384- to 398-positions and that an amino acid(s) mutates into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions and the 41- to 47-positions.
- (4) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 384- to 398-positions and that an amino acid(s) mutates into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions and the 125- to 133-positions.
- (5) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 384- to 398-positions and that an amino acid(s) mutates into another amino acid(s) at a region(s) selected from the group consisting of the 41- to 47-positions and the 125- to 133-positions.
- (6) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 11- to 14-positions and that an amino acid(s) mutates into another amino acid(s) at the 384- to 398-positions.
- (7) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 41- to 47-positions and that an amino acid(s) mutates into another amino acid(s) at the 384- to 398-positions.

- (8) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 125- to 133-positions and that an amino acid(s) mutates into another amino acid(s) at the 384- to 398-positions.
- (9) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 384- to 398-positions.
- (10) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that an amino acid(s) at the 384- to 398-positions mutates into another amino acid(s) selected from the group consisting of Ala, Pro, Leu, Val, Gly, Arg, Glu and Phe and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41- to 47-positions and the 125- to 133-positions.
- (11) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that an amino acid(s) at the 390- to 392-positions mutates into another amino acid(s) selected from the group consisting of Ala, Pro, Leu, Val and Phe and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41- to 47-positions and the 125- to 133-positions.
- (12) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that Gly at the 392-position mutates into Pro and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41- to 47-positions and the 125- to 133-positions.
- (13) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that a mutation selected from the group consisting of a mutation of IIe at the 390- position into AIa, a mutation of AIa at the 391- position into Phe, Val or Leu and a mutation of AII at the 392-position into Pro is present and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41-to 47-positions and the 125- to 133-positions.
- (14) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that a mutation selected from the group consisting of a mutation of Ala at the 384- position into Gly, a mutation of Ala at the 387- position into Phe, a mutation of Val at the 389-position into Pro, a mutation of Pro at the 397- position into Arg and a mutation of Asn at the 398-position into Glu or Alg is present and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41- to 47-positions and the 125- to 133-positions.

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- (15) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that a mutation selected from the group consisting of a mutation of Lys at the 11- position into IIe, a mutation of Asp at the 14- position into Ser is present and that an amino acid-(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 41- to 47-positions, the 125- to 133-positions and the 384- to 398-positions.
- (16) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that a mutation selected from the group consisting of a mutation of Lys at the 11- position into IIe and a mutation of Asp at the 14- position into Ser, and, another mutation selected from the group consisting of a mutation of IIe at the 390- position into Ala, a mutation of Ala at the 391-position into Phe, Val or Leu and a mutation of Gly at the 392-position into Pro are present, and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 41- to 47-positions and the 125- to 133-positions.
- (17) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that a mutation selected from the group consisting of a mutation of Lys at the 125- position into Gln, a mutation of Arg at the 132-position into Gln and a mutation of Lys at the 133- position into Gln and a mutation of Lys at the 133- position into Asn or Gln is present and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41- to 47-positions and the 384- to 398-positions.
- (18) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that a mutation selected from the group consisting of a mutation of Lys at the 125- position into Gln, a mutation of Arg at the 132-position into Gln and a mutation of Lys at the 133- position into Asn or Gln, and, another mutation selected from the group consisting of a mutation of IIe at the 390- position into Ala, a mutation of Ala at the 391- position into Phe, Val or Leu and a mutation of Gly at the 392-position into Pro are present, and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group

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consisting of the 11- to 14-positions and the 41- to 47-positions.

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- (19) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that Gly at the 392-position mutates into Pro.
- (20) A human AT III mulant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that Ala-Gly at the 391- to 392-positions mutates into Phe-Pro.
- (21) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that IIe-Ala at the 390- to 391-positions mutates into Ala-Leu.
- (22) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that Lys at the 125-position mutates into Gln and Ala-Gly at the 391- to 392-positions mutates into Phe-Pro.
- (23) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that Arg-Lys at the 132- to 133-positions mutates into Gln-Asn and Ile-Ala at the 390- to 391-positions mutates into Ala-Leu.
- (24) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that Arg-Lys at the 132- to 133-positions mutates into Gln-Asn and Ala-Gly at the 391- to 392-positions mutates into Phe-Pro.
- (25) A human AT III mutant obtained by subjecting human AT III to mutation, which has human AT III amino acid sequence except that Lys at the 133-position mutates into Asn and Ala-Gly at the 391- to 392-positions mutates into Phe-Pro.
- The present invention includes human AT III mutants which are obtained by substituting an amino acid-(s) constituting natural human AT III with another amino acid(s) at a desired position(s).

Each of these human AT III mutants is expressed and produced by using animal cells as a host. As will be described hereinbelow, the mutants thus obtained exhibit elevated antithrombin activities in the absence of heparin as compared with a plasma derived human AT III concentrate or a natural recombinant human AT III. Further, these mutants exert improved drug efficacys in tests with the use of animals as compared with the plasma derived human AT III concentrate. Thus it is expected that they are highly useful for clinical purposes.

The present invention also relates to a DNA coding for the human AT III mutant according to the present invention, an expressible vector which has a DNA containing part or the whole of the DNA sequence coding for the human AT III mutant according to the present invention, a transformant which is obtained by subjecting host cells to transformation with the above-described expressible vector and a method for producing a human AT III mutant which comprises incubating the above-described transformant and recovering the human AT III mutant produced by the transformant from the culture.

The present invention further relates to a drug composition for thrombotic disorders which contains the human AT III mutant according to the present invention and pharmaceutically acceptable carriers, a use of the human AT III mutant according to the present invention for the making of a medicament for treating thrombotic disorders, and a method for treating thrombotic disorders which comprises administering a pharmaceutically effective amount of the human AT III mutant according to the present invention to a patient suffering from the thrombotic disorders.

Further scope and the applicability of the present invention will become apparent from the detailed description given hereinafter. However, it should be understood that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

The present invention will be described hereinafter in detail.

The term "AT III" means human AT III in the following description.

Detailed Description of the Invention

1) Isolation of cDNA coding for AT III

Since AT III is mainly synthesized in the liver, a commercially available human liver cDNA library (\lambdagt 11, available from Clonetech) may be used for the isolation of cDNA coding for AT III. Cloning can be effected by a publicly known method. For example, the plaque hybridization method with the use of a synthetic oligonucleotide corresponding to AT III amino acid sequence as a probe [see Sambrook, J. et al., Molecular Cloning, Cold Spring Harbor Laboratory (1989)] may be used therefor.

The clones thus obtained are subcloned into a plasmid such as pUC 18, if required. The nucleotide sequence of cDNA thus obtained can be determined and estimated by the Maxam-Gilbert method (see

Maxam, A.M. and Gilbert, W., Proc. Natl. Acad. Sci. USA. 74, 560 (1977)) or the dideoxy method (Sanger, F., Science, 214, 1205 (1981)). The nucleotide sequence of the coding region of AT III cDNA thus obtained and the amino acid sequence deduced therefrom are given in SEQ ID. No. 1 in the sequence listing. The amio acid sequence was also described in SEQ ID No. 2 in sequence listing.

2) Method for site-directed mutagenesis

Examples of the method for site-directed mutagenesis include a method of Zoller et al. [see Zoller, M. and Smith, M. Methods in Enzymology, 100, 468 (1983)], the one of Kramer et al. [see Kramer, W. and Fritz, H-J, Methods in Enzymology, 154, 350 (1987)] and the one of Vandeyar et al. [see Vandeyar et al., Gene, 65, 129 (1988)].

In the method of Kramer et al., which is called the gapped duplex method, amber mutants of M13 phage such as M13tv18 and M13tv19 are usable as a vector. A DNA coding for AT III is cloned into these vectors. The single-stranded DNA thus obtained and a double-stranded DNA fragment of M13 free from amber mutation (a vector fragment obtained by cleaving M13mpP with Pvu II) are denatured and subjected to degenerative annealing to thereby give a gapped duplex DNA. Next, this DNA is hybridized with a synthetic oligonucleotide having the mutation to be introduced thereinto. After filling up the gap by treating with DNA polymerase and DNA ligase, it was transfected into E. coli mutS strain (BMH71-18 mutS). Then a nonamber phage capable of growing exclusively in supO E. coli is selected. Thus a phage having the desired mutation introduced thereinto can be efficiently obtained. In a practical operation, a commercially available kit (Mutan-G, manufactured by Takara Shuzo Co., Ltd.) may be used. On the other hand, the method of Vandeyar et al. is effected as follows. A single-stranded DNA of M13, into which a DNA.coding for AT III has been cloned, is hybridized with an oligonucleotide having the mutation to be introduced: By using it as a template, dATP, dGTP, dTTP and 5-methyl-dCTP are used as substrates and treated with T7 DNA polymerase. The double-stranded DNA thus formed is treated with T4 DNA ligase to thereby give a closed-circular double-stranded DNA. Next, this double-stranded DNA is treated with a restriction enzyme Msp1 and then with exonuclease III. Thus a circular single-stranded DNA exclusively consisting of a strand having the mutation introduced thereinto is obtained. Then it is transfected into an E. coli (SDM strain) free from any restriction system specific for methylated DNA. Thus the desired clone can be efficiently obtained. In the case of this method, a commercially available kit may be used in practice (T7-GEN In Vitro Mutagenesis Kit, manufactured by United States Biochemical Corporation). The synthetic oligonucleotide having the mutation to be introduced can be synthesized by the phosphoramidite method with the use of a DNA synthesizer (Model 380 A, manufactured by ABI).

3) Preparation of template for introducing AT III cDNA mutation

A template for introducing mutation is prepared by inserting restriction sites before and after the coding region of the AT III cDNA obtained in the item 1). The restriction enzymes may be selected from among publicly known ones. In the case of the present invention, a Hind III restriction site was inserted immediately before the coding region of the AT III cDNA while a BgI II restriction site was inserted immediately thereafter.

First, a plasmid containing the AT III cDNA obtained in the item 1) described above is cleaved with EcoR I and thus a fragment of 1.5 kb including the whole AT III coding region is obtained. This fragment is inserted into a linearized product obtained by cleaving the RF (Replicative Form, a double-stranded DNA) of phage M13tv18 with EcoR I.

Among the clones thus obtained, a single-stranded DNA containing the sense strand of AT III is used as a template. In accordance with the method of Kramer et al., two synthetic oligonucleotides containing the restriction sites of Hind III and BgI II respectively are used as primers and the restriction sites are inserted before and after the coding region of the AT III cDNA.

Subsequently, a fragment containing the AT III cDNA sequence obtained from the clone is inserted into an appropriate plasmid to thereby constract a template for introducing mutation.

In the case of the present invention, a template for introducing mutation can be prepared by inserting the DNA fragment of about 1.5 kb containing the whole AT III coding region, which is obtained by cleaving the above-mentioned clone with Hind III and EcoR I, into the plasmid M13tv19 RF or M13mp19 cleaved with the same enzymes.

Further, the AT III cDNA has a Sac I restriction site (the base part at the 721- to 726-positions in SEQ. ID No. 1) whereby the reactive site can be separated from the heparin binding site. Accordingly, the N-terminal side of AT III obtained by cleaving the above-mentioned clone with Hind III and Sac I, namely, the

DNA fragment containing the heparin binding site is insered into the plasmid M13tv19 or M13mp19 cleaved with the same enzymes. Thus a template for introducing mutation into the heparin binding site can be prepared.

Regarding the reactive site, a similar operation can be carried out by using EcoR I and Sac I.

4) Introduction of mutation into the desired site

In the amino acid sequence of AT III, an amino acid at a desired position can be mutated into another desired amino acid (hereinafter referred to as the desired amino acid) in accordance with the above-mentioned publicly known methods by using a synthetic oligonucleotide containing a DNA coding for the desired amino acid and an appropriate plasmid described in the item 3) as a template. When Gly at the 392-position in AT III is to be mutated into Pro, for example, the AT1R oligonucleotide given in Table 1 may be used. In order to mutate Ala-Gly at the 391-to 392-positions into Phe-Pro, the AT5R oligonucleotide listed in Table 1 may be used. When a number of amino acids separately located are to be mutated, a number of mutations can be introduced by successively effecting the operations for introducing the mutations one by one.

Typical examples of oligonucleotides employed in the present invention are listed in Tables 1 and 2. Amino acid mutation positions and desired amino acids are listed in Tables 3 and 4. Base codons coding for the desired amino acids are not restricted to those listed in Tables 1 and 2 but any codon may be used therefor so long as it codes for the desired amino acid.

Table 1

Nucleotide sequence of synthetic oligonucleotide for introducing AT III mutation, amino acid to be mutated and position thereof

Oligonucleotide		Nucleotide sequence	Amino acid to be mutated and its position
ATIR	5	GTTTAGCGACCGCGGAGCAATCAC 3'	Gly392 -Pro
ATSR	ິນ	GGGGTTTAGCGACCGCGGGAAAATCACAACAGC 3'	Ala391 -Phe Gly392 -Pro
A'I'7R	įù	TAGCGAACGGCCGACAGCAACAGCGGT 3'	Ile390 -Ala Ala391 -Val
AT9R	5.	CAGCGGTACTGCCAGCTGCTTC 3'	Ala384 -Gly
AT19R	ິນ	ACGCCCAGCAATCGGAACAGCGGTACT 3'	Val389 -Pro
AT24R	ູນ	AATCACAACAAGGTACTTGCAG 3'	Ala387 -Phe
AT'26R	3	GTTTAGCGAACGCGGAADAATCACAACAGC 3'	Ala391 -Ile Gly392 -Pro
AT27R	ີ່ເວ	GTTTAGCGAACGCGGACCAATCACAACAG 3'	Ala391 -Gly Gly392 -Pro
AT28R	ູນ	GITTAGCGAACG <u>CGGATAAA</u> TCACAACAGC 3'	Ala391 -Tyr Gly392 -Pro
AT29R	S	GTTTAGCGAACGCGGCCAAATCACAACAGG 3'	Ala391 -Trp Gly392 -Pro
A1'30R	5	GITTAGCGAACG <u>CGGAAC</u> AATCACAACAG 3'	Ala391 -Val Glv392 -Pro
AT34R	5	TACCGAACGGCCAATAGGCACAACAGCGGT 3.	Ile390 -Ala Ala391 -Ile
AT35R	ເລ	TAGCGAACGGCCAAGAGCACAACAGCGGT 3'	Ile390 -Ala Ala391 -Leu
AT38R	5	TAGCGAACGGCC <u>AAGACC</u> CACAACAGGGG 3'	Ile390 -Gly Ala391 -Leu
AT39R .	5	GTTTAGCGAACG <u>GGGAACAGC</u> CACAACAGCGGTA 3'	Ile390 -Ala Ala391 -Val Gly392 -Fro

The underlined part in the nucleotide sequence represents the sequence corresponding to the amino acid to be mutated.

Nucleotide sequence of synthetic oligonucleotide for introducing AT ill mutation, amino acid to be mutated and position thereof

		-
Oligonucleotide	Nucleotide sequence	Amino acid to be mutated and its position
AT40R	5' GTTTAGCGAACGGGAAAAAGCACACACACACACACACACA	
AT46R	5' GTTTAGCGAACGCGGAACAATTACAACAACAA	ile390 -Leu Ala391 -Phe Gly392 -Pro
AT48R	5' GTTTAGCGAACGCGGATAAGCCACAAACAACAACAAAAAAAA	Alassı -Leu Gly392 -Pro
AT49R	5' GTTTAGCGAACGCGGCCAAACCAAAAAAAAAAAAAAAAA	Tleasu -Ala Alassi -Tyr Glyss2 -Pro
ATSOR	5' GTTTAGCGAACGCGCCAAAGCACAAACCAACAAAAAAAAA	Tiessu -Ara Alassi -Trp Glyss2 -Pro
AT2R'	5' GAAAGTCACCTCTCGGGGTTTAGCCAAC	iledsu -Leu Aladsi -Trp Gly392 -Pro
ATSR'	5' TTGAAAGTCACCTCCTCGGTTTACCCAAC 3	Asnaya -Glu
AT6R'	5' TTGAAAGTCACCCGTCGACGTTTTACCCAACG 3	ASDASS TALE
· ATIG	5' CGGCAGTTCAGTTGGCAAAGAAGAACAAA	Frose/ -Arg Asn398 -Arg
AT2G	5' GGATTIGITGCGTTTTGATAGAGTCCCA 3'	Lys125 ~61n
AT7G	5' GATAGAGTTGGCAGTTCAG 3'	Areton of a
ATBG	5' GGTGGCCTCCAGGATCTTCTG 3'	71 8129 -011 Dr. 41 1011
AT96	5' GGGATTCATGGGAATGGATCGTGGGATTCCTCTACAT '3	rro 41 -Leu
ATIF	5' GTTGGCTTTTTTGATAGAGTCC 3'	Lys 11 -11e Asp 14 -Ser
AT2F	5 TITIGITEGERITATION SI	Argisz -Gin
AT3F	5' TTTGTTGGCTTGTCGATAGAG 3	Lys133 = Asn Lys133 = Glo
		CTC CTI

The underlined part in the nucleotide sequence represents the sequence corresponding to the amino acid to be mutated.

			398 Pro Asu	0.0	1000				Pro Asn	Pro Asn	Pro Asn	Pro Asıı	Pro Asn	Pro Asu	Pre Asu	Pro Asıı	Fro Asu	Pro Asıı	Pro Asu	Pro Asn	Pro Asn	Pre Asu	Pro Asn		Pro Are	
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		acid in AT III mutant	Ser T	Ser T	Ser T	Ser T	Ser T	Ser T	Ser T	Ser Ti	-		-			-						-	Ser Ti	Ser TI	Ser TI	Ser T
25	J.e 3	d In	384 Ala S	Ala S	Alu S	Ala S	Ala S	Ala S	Ala S	Alu S	5										S XIO	Ala s	Alu S	Ala S	Ala S	Ala S
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III mutant	Ala Val	
S Table 4	384 Alu Ser Thr	Ala Ser Thr
S Tr Mutated amino a	132 133 Arg Lys	
<i>35</i>	129 Arg -	A Arg
40	14 125 - Asp Lys	Asp Gln Asp Gln Asp Gln Asp Gln Asp Lys Asp
45	1	Lys
50	Natural AT 111	1638 1638 1638 2638 2638 2638 2638 1658 3658 3658 3658 3658 3658 16358 12638 12638 12638 12638 12638

55 5) Combination of mutation in the neighborhood of reactive site and mutation at heparin binding site

As described above, AT III cDNA involves a Sac I rostriction site which is located between the reactive site and the heparin binding site. Thus a fragment containing the heparin binding site and another one

containing the reactive site can be obtained by cleaving a plasmid containing the mutated AT III DNA obtained by the method described in the aforementioned item 4) with Hind III and SacI or Sac I and EgI II. An AT III mutant DNA, in which both of the reactive and heparin binding sites have mutated, can be prepared by treating an AT III DNA having a mutated reactive site and another AT III DNA having a mutated heparin binding site, respectively, with restriction enzymes to thereby give a DNA fragment having a mutated reactive site and another DNA fragment having a mutated heparin binding site and connecting the mutated DNA fragments with an appropriate plasmid. According to this method, any combination of mutations at these sites can be achieved. Any plasmid can be used as the one to which the mutated DNA fragments are connected so long as it is suitable for the expression thereof in a host. For example, pSV2 and pK4K are usable.

In Table 4, a symbol 2G35R means a mutant obtained by combining a 2G-mutated DNA fragment with a 35R-mutated one.

6) AT III mutant recombinant expression vector and transformant thereof

The DNA coding for the AT III mutant obtained by the above-mentioned method is inserted into an appropriate vector and then the vector obtained is transfected into appropriate host cells. Thus a transformant can be obtained. This transformant is incubated by a conventional method and thus an AT III mutant can be produced in a large amount from the culture.

A DNA coding for an AT III mutant is reconnected to a vector suitable for the expression of the AT III mutant at the downstream of the promoter of the vector by a publicly known method with the use of a restriction enzyme and DNA ligase. Thus a recombinant expression vector can be constructed. The vector is not particularly restricted, so long as it can be replicated and amplified in a host. Neither the promoter nor the terminator is particularly restricted too, so long as they correspond to the host to be used in the expression of the nucleotide sequence coding for the AT III mutant. Thus an appropriate combination thereof may be selected depending on the employed host.

The recombinant expression vector thus obtained is transfected into a host by the competent cell method [see Hanahan, D., J. Mol. Biol., 166, 557 (1983)], the calcium phosphate method [see Wigler, M. et. al., Cell, 11, 222(1977)] and so on to thereby form a transformant. As the host, E. coli, animal cells, etc. are usable. The transformant thus obtained is incubated in a medium suitable for the host. The incubation may be usually carried out at a temperature of from 20 to 45 °C at a pH value of from 5 to 8 with aeration and stirring, if necessary. The AT III mutant can be separated and purified from the culture by combining publicly known separation and purification methods. Examples of these publicly known methods include salting out, solvent precipitation, dialysis, gel filtration, electrophoresis, ion exchange chromatography, affinity chromatography and reversed phase high performance liquid chromatography. The AT III mutant thus obtained has an elevated antithrombin activity in the absence of heparin and an elevated in vivo antithrombotic action in rat as each compared with natural AT III.

Effects of the Invention

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(1) Antithrombin activity

By using a Testzym AT III 2 Kit (manufactured by Daiichi Kagaku Yakuhin), the antithrombin activity of the AT III mutant according to the present invention was measured. Namely, the inhibition activity on thrombin thereof in the absence of heparin was measured by using a synthetic substrate (S-2238) of thrombin. As a control, a plasma derived AT III concentrate (Anthrobin P; manufactured by Hoechst Japan) was employed.

In this measurement, a 50 mM Tris hydrochloride buffer solution (pH 7.5) containing 0.1% of bovine serum albumin and 0.15 M of sodium chloride was used. Specimens of various concentrations were reacted with a given amount of thrombin (originated in bovine) at 37 °C for 5 minutes. After the completion of the reaction, the synthetic substrate S-2238 was added and the amount of p-nitroaniline liberated for 2 minutes was determined based on a change in the absorbance at a wavelength of 405 nm. Thus, the remaining thrombin activity was measured. Under these conditions, the AT III mutant concentration at which 50% of the thrombin activity was inhibited (hereinafter referred to as the IC_{50}) was calculated.

Table 5 shows the IC_{50} values of mutants. The IC_{50} of the plasma derived AT III concentrate in the absence of heparin was 13.0×10^{-2} M and that of the natural recombinant AT III was on almost the same level. In contrast, the IC_{50} values of the AT III mutants of the present invention were clearly lower than them, suggesting that the antithrombin activity in the absence of heparin had been elevated.

Table 5

		Antithrombin activ	ity of AT III mu	tant
5	Specimen	Antithrombin activity IC _{50 × 10⁻⁸ (M)}	Specimen	Antithrombin activity IC50×10 ⁻² (M)
10	AT III concentrate Natural recombinant AT III 1R	13.0 14.0 3.0		
	5R 26R 27R 28R	1.7 3.1 8.2 2.8	38R 9R 19R	6.1 5.8 8.7
15	29R 30R 46R	2.6 1.8 2.3 5.0	24R 2R' 5R' 1G1R	10.0 3.8 4.7
	39R 40R 48R	5.6 3.1 5.7	1G5R 2G1R	3.7 2.9 3.8
20	49R 50R 7B	5.7 5.6 3.0 2.9	2G5R 2G30R 2G35R 7G5R	2.9 1.6 2.2
25	34R 35R	3.5 3.5	9G5R 127G5R	1.8 1.7 1.5

(2) Affinity for heparin

The affinities for heparin of the AT III mutants according to the present invention were compared and examined by the high performance liquid chromatography method with the use of Heparin-5PW (7.5 mm x 75 mm; manufactured by Tosoh Corp.). Namely, a 50 mM Tris hydrochloride buffer solution (pH 7.5) was used as a mobile phase and the concentration of sodium chloride was linearly increased from 0 M to 2 M within 30 minutes at a flow rate of 1 ml/min. The detection was effected based on the absorption at a wavelength of 280 nm and the time required for the elution of each specimen was compared.

As Table 6 shows, the main peak fractions of the AT III and the natural recombinant AT III were eluted, respectively, 22.3 minutes and 23.1 minutes after the intiation of the elution, showing no large difference. Compared with the AT III and the natural recombinant AT III, the mutants having a mutation in the neighborhood of the reactive site showed no remarkable difference. On the other hand, the mutants having mutations in the neighborhood of the reactive site and at the heparin binding site showed each a significantly shortened elution time of the main peak fraction. It was thus confirmed that the introduction of a mutation into the heparin binding site would have lowered the affinity for heparin.

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Table 6

Specimen	Elution time (min)	Specimen	Elution time (min)
AT III concentrate	22.3	-	
Natural recombinant AT III	23.1		
5R	21.4	9R	22.5
26R	21.2	19R	23.2
28R	22.5	24R	23.4
29R	21.0	5R'	23.6
30R	20.4	1G1R	14.0
46R	17.4	1G5R	14.3
40R	21.0	2G1R	12.5
48R	21.6	2G5R	12.9
7R	21.9	2G30R	13.0
35R	22.1	2G35R	13.1
38R	21.9	7G5R	12.9
	}	127G5R	10.2

(3) Antithrombotic action of AT III mutant

By using a plasma derived AT III concentrate (Anthrobin P; manufactured by Hoechst Japan)...and a natural recombinant AT III as controls, the antithrombotic actions of the AT III mutants according to the present invention were measured by the following method.

A method reported by Peters et al. [see Peters, R.F. et al., Thrombosis Haemostasis, 65, 268 (1991)] was modified and employed. Namely, a shunt was formed by cannulating Atom Venous Catheter (4Fr, 3.5 cm, manufactured by Atom) filled with a physiological saline into the carotid arteriovein of a male Sprague-Dawley rat (200 - 300 g) under anesthesia. After blocking the blood stream, the artery side of the shunt was provided with a pulse wave pickup (MPP-3, manufactured by Nippon Koden) and thus changes in the blood stream were monitored with a polygraph recorder during the test period. A calculated amount of a specimen material was diluted with a physiological saline to give a volume of 1 ml and quickly administered once to the rat via the femoral vein. Then the shunt was opened and the blood was allowed to pass. The time required from the point of opening the shunt to the point of the occlusion of the shunt due to the formation of a thrombus was measured and defined as the occlusion time.

Tables 7 and 8 show the results. It was thus proved that the AT III mutants of the present invention had strong antithrombotic actions as compared with the plasma derived AT III concentrate and the natural recombinant AT III.

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Table 7

		Antithrombotic ac	tion of AT III mutant	
5	Specimen	Dose (mg/kg)	Occlusion time Mean ± SD (min)	Case no.
	Physiological saline		21.4± 2.7	11
	AT III concentrate	8	29.1± 8.0	9
	ł	16	36.4±11.6	8
10		32	46,6±14.3	8
	Natural recombinant AT III	16	39.3±10.5	6
		32	49.0±13.7	4
	5R	8	46.0±18.6	7
15		16	65.7±21.0	6
	30R	4	35.3± 7.1	6
		8	43.6± 4.7	6
		16	52.2± 5.3	6
20	35R	2	34.7± 5.8	7
		4	39.9± 9.4	7
		8	61.4±12.6	7
	1G5R	4	34.1± 8.7	8
25	. 1	8	45.2±10.1	6
		16	69.0±25.7	6
	2G5R	4	45.7± 7.5	7
	ļ	8	53.6± 9.3	9
30		16	70.6±11.5	8
.;~-	2G30R	4	35.5± 5.5	6
•		8	45.7±11.2	6
•••		16	53.8±13.7	6
	2G35R	2 .	43.8± 6.6	6
35		4	45.2± 5.8	6
		8	62.7±28.2	6

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Table 8

	Antithrom	botic action of AT III mutant	
Specimen	Dose (mg/kg)	Occlusion time Mean ± SD (min)	Case no.
1F5R	4	38.3= 6.0	6
	8	41.3± 7.1	6
	16	54.7±13.1	6
2F5R	4	39.5= 6.1	6
	8	47.8± 9.5	6
	16	. 59.8±16.1	6
3F5R	4	38.5± 6.3	6
į	8	45.3± 5.2	6
ŀ	16	55.7± 4.5	6
7G5R	4	36.5± 5.1	6
	8	39.7± 3.9	6
	16	54.2±18.3	6
G5R	2	38.3± 2.7	6
1	. 4	38.5± 3.1	6
	. 8	49.2± 2.8	6
2G5R	2	36.5± 6.0	6
	4	43.7± 2.7	6
	8	51.2± 6.3	6
27G5R	2	36.0± 7.4	6
ļ	4	46.8± 4.4	6
ì	8	57.0±10.9	6

These results suggest that the AT III mutants according to the present invention serve as anticoagulants and suppress the formation of thrombi. Thus there are expected to be useful as preventive and therapeutic agents for thrombotic disorders.

(4) Effect of AT III mutant on experimental model of disseminated intravascular coagulation (DIC)

By using a plasma derived AT III concentrate as a control, the effects of the AT III mutants according to the present invention on an experimental model of disseminated intravascular coagulation (DIC) were examined by the following method. A method reported by Sugishima et al., [see Tadashi Sugishima et al., Rinsho to Kenkyu, 62, 274 (1985)] was modified and employed. Namely, a model was formed by cannulating an Atom Venous Catheter (3Fr, manufactured by Atom) into the jugular vein of a male Sprague-Dawley rat (200 - 300 g) under anesthesia and continuously administering tissue thromboplastin (Thromborel S, manufactured by Behringwerke, AG) for an hour. A test specimen was rapidly administered once via the femoral artery of the rat immediately before starting the administration of tissue thromboplastin. Thirty minutes after the completion of the administration of tissue thromboplastin, the blood was sampled via the descending aorta of the ral and 1/10 volume of 3.8 % sodium citrate was added thereto. After the sampling, 0.5 ml of the blood was immediately taken in a container for an automatic hemocytometer (manufactured by Toa Iyo Denshi K.K.) and platelets were counted with an H.1 System (manufactured by Technicon). The residual blood was centrifuged (3000 rpm, 10 min) to thereby give the plasma. Then fibrinogen contained in the plasma was determined. The content of fibrinogen in the plasma was measured by the thrombin time method (Fibrinogen B-Test Wako, manufactured by Wako Pure Chemical Industries, Ltd.).

Table 9 shows the results. Thus it was found out that the AT III mutants of the present invention exerted strong effects on a decrease in platelet count and the reduction of plasma fibrinogen level in the experimental DIC model induced with tissue thromboplastin as compared with the plasma derived AT III concentrate. Based on these results, the AT III mutants of the present invention are expected as a useful therapeutic agent for DIC.

Table 9

Effect of AT	III mutant o	n experim	ental DIC model	
Specimen	Dose (mg/kg)	No. of cases	Platelet count (x 10 ³ /µl) Mean ± SD	Amount of plasma fibrinogen (g/l) Mean ± SD
Physiological saline (no tissue thromboplastin administered)		12	952.7±110.6	1.95±0.15
Sole administration of tissue thromboplastin		12	424.1±122.3	0.12±0.03
AT III concentrate	8	12	527.0±108.8	0.17±0.06
	16	11	596.6± 60.9	0.20±0.07
	32	12	683.7±128.9	0.77±0.41
1G5R	4	6	574.7± 54.2	0.36±0.42
	8	6	729.7± 77.6	0.99±0.54
2G5R	4	6	618.5±116.1	0.21±0.07
	8	6	618.2±146.3	0.77±0.28
1F5R	4	6	557.2±154.4	0.30±0.34
	8	6	649.5±112.6	0.64±0.39
2F5R	4	6	528.8± 89.1	0.24±0.08
	8	5	659.8± 53.6	0.63±0.24
3F5R	4	6	487.3± 83.4	0.16±0.08
	8	6	664.5± 61.5	0.54±0.37

This AT III mutant can be orally, topically, intravenously, intramuscularly or subcutaneously administered, among which topical or intravenous administration is preferable. The dose may range from 0.1 to 100 mg/kg and preferably from 0.5 to 20 mg/kg, and is determined depending on the body weight of the patient. It is dissolved in from 1 to 50 ml of a physiological saline and used.

It may be formulated into, for example, wettable powders, solutions, tablets, capsules, powders, suppositories and the like. As carriers for formulating these preparations, pharmaceutically acceptable fillers, disintegrating agents, lubricants and dispersion media commonly employed in the art may be used.

Brief Description of the Drawings

Fig. 1 is a figure showing a process for constructing pKCRNK.

Fig. 2 is a figure showing a process for constructing pUC19st-Ad.

Fig. 3 is a figure showing a process for constructing pAdPst-.

Fig. 4 is a figure showing a process for constructing pKCRNKAd.

Fig. 5 is a figure showing a process for constructing pKCR5H3B.

Fig. 6 is a figure showing a process for constructing pKCR5H3BAd.

Fig. 7 is a figure showing a process for constructing pKCRAdEcoB⁻H⁻.

Fig. 8 is a figure showing a process for constructing pKNK.

Fig. 9 is a figure showing a process for constructing pK4K.

Fig. 10 is a figure showing a process for constructing pKCR5RAd.

Examples

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To further illustrate the present invention in detail and concretely, the following Examples will be given, though it is to be understood here that the present invention is never restricted thereto.

Example 1

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Cloning of DINA sequence coding for AT III

By the use of a commercially available human liver cDNA library (\(\lambda\gamma\) 11, available from Clonetech) as a starting material, screening was effected by a conventional method with a ³²P-labeled synthetic oligonucleotide as a probe. The sequence of the synthetic oligonucleotide comprised the nucleotide sequence corresponding to the amino acids at the 314- to 322-positions of AT III based on the report by Chandra et al.

As the result of the screening, two clones #2 and #6 were obtained. DNA fragments were collected from each clone by using a restriction enzyme EcoR I and subcloned into M13mp18 to thereby determine the nucleotide sequence. As a result, it was confirmed that the clone #2 contained a fragment of about 1.3 kb corresponding to the sequence of the 33rd amino acid to polyA, while another clone #6 contained a fragment of about 1.1 kb corresponding to the initiation codon to the 348th amino acid. Subsequently, inserts were excised from these clones by using EcoR I and each of the inserts was subcloned into pUC18 cleaved with EcoR I. Thus pUC-H and pUC-L were prepared respectively from the clones #2 and #6.

Next, a DNA fragment of about 3.7 kb (containing a sequence of about 1.0 kb corresponding to pUC18 and the N-terminal side of antithrombin III), which was obtained by cleaving pUC-L with Nco I and Hind III, was connected to another DNA fragment of about 0.5 kb (containing a sequence corresponding to the C-terminal side of antithrombin III) which was obtained by cleaving pUC-H with Nco I and Hind III. Thus a plasmid AT III FLpUC containing all coding regions ranging from the initiation codon to the terminator codon of AT III was obtained. The whole sequence from the initiation codon to the terminator codon of the AT III cDNA contained in this plasmid was represented by SEQ ID No. 1 in the sequence listing.

Example 2

Insertion of restriction site

By the use of the plasmid AT III FLpUC obtained in the above Example 1 as a starting material, a DNA having a Hind III restriction site inserted immediately before the AT III coding sequence and a BgI II restriction site immediately thereafter was prepared. First, AT III FLpUC was cleaved with EcoR I to thereby give a fragment of about 1.5 kb containing the whole AT III coding region. This fragment was inserted into the above-mentioned one obtained by cleaving RF of M13tv18 with EcoR I to linearize. Among the clones thus obtained, a clone giving the sense strand of AT III as a single-stranded DNA was referred to as tvATR. By using the single-stranded DNA of this tvATR as a template and the two synthetic oligonucleotides given below, each containing the restriction site of each enzyme, as a primer, the restriction sites were introduced in accordance with the method of Kramer et al.

5' TACATGGCCGAAGCTTCGTAATCAT 3'.

AT3B 29mer:

5' CAAAGAATAAGATCTTATTACTTAACACA 3'.

In the practical operation, a commercially available kit (Mutan G, manufactured by Takara Shuzo Co., Ltd.) was used. Namely, about 0.5 µg of the single-stranded DNA of tvATR and 0.2 µg of dsDNA contained in the kit (obtained by cleaving the RF DNA of a phage M13mpP lacking in a Pvu II fragment containing the multiple-cloning site of M13mp18 with Pvu II to linearize) were allowed to stand in 20 mM Tris-HCl pH 8 -10 mM MgCl₂ - 50 mM NaCl - 1 mM DTT at 100 °C for 3 minutes, at 65 °C for 10 minutes and at 37 °C for 10 minutes to thereby form a gapped duplex. A 1/10 portion of this gapped duplex was collected and mixed with 5 pmol portions of AT5H and AT3B the 5'-end of which had been substituted with phosphate with T4 polynucleotide kinase, and the resulting mixture (3 µl in total) was allowed to stand at 65 °C for 15 minutes and at 37 °C for 15 minutes. Next, 25 µI of a buffer solution contained in the kit [50 mM Tris • HCl pH 8 - 60 mM ammonium acetate - 5 mM MgCl₂ - 5 mM DTT - 1 mM NAD - 0.5 mM each of dNTPs (A, C, G, T)], 60 U of E. coli DNA ligase and 1 U of T4 DNA polymerase were added thereto and the resulting mixture was allowed to stand at 25 °C for about 2 hours. After adding 3 µl of 0.2 M EDTA (pH 8) and heating at 65 °C for 5 minutes, part of the mixture was collected and transfected into competent cells of an E. coli BMH71-18mutS strain prepared by the method of Hanahan [see Hanahan, D., J. Mol. Biol., 166, 557 (1983)]. Plaques obtained by using an E. coli MV1184 strain as an indicator were picked and incubated by a conventional method to thereby give an RF DNA. This DNA was cleaved with restriction enzymes Hind III and Bgl II and the nucleotide sequence of a clone having a new restriction site was determined by the

dideoxy method. Thus it was confirmed that the desired mutation had been introduced. The clone thus obtained was referred to as AT5H3B.

A DNA fragment of about 1.5 kb obtained by cleaving this AT5H3B with Hind III and EcoR I was inserted into M13lv19RF which had been subjected to linearize by similarly cleaving with Hind III and EcoR I. The clone thus obtained was referred to as tv19-5H3B. A DNA fragment obtained by cleaving a plasmid pSV2-dhfr [see Lee, F. et al., Nature, 294, 228 (1981); Subramani, S. et al., Mol. Cell. Biol., 1, 854 (1981)] with Hind III and Bgl II and eliminating a region coding for mouse dihydrofolate reductase (dhfr) was connected to another DNA fragment obtained by cleaving AT5H3B with Hind III and Bgl II too and containing the whole AT III coding region. Thus a plasmid pSV2-5H3B was obtained. Further, a DNA fragment of about 730 bp obtained by cleaving pSV2-5H3B with Hind III and Sac I and coding for the N-terminal side of AT III was inserted into M13tv19 and M13mp19 which had been subjected to linearize by cleaving with Hind III and Sac I to thereby respectively give tv19-ATN and mp19-ATN.

Example 3

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a) Preparation of 1R mutant DNA

A sequence coding for an AT III mutant 1R wherein the 392nd Gly of AT III had been substituted with Pro (Table 3) was obtained by the site-directed mutagenesis method. Namely, in accordance with the method of Kramer et al., the single-stranded DNA of AT5H3B obtained in Example 2 was used as a template and treated with a synthetic oligonucleotide AT1R (Table 1) to thereby give the desired clone 1Rmut. The operation was effected by using a commercially available kit (Mutan G) by the same method as the one described in Example 2.

Twelve plaques thus obtained were picked up and analyzed. As a result, five of these clones were found to be the desired ones. The RF DNA of the obtained clone was cleaved with Hind III and Bgl II and the DNA fragment of about 1.4 kb thus obtained was replaced with a mouse DHFR gene in a plasmid pSV2-dhfr, similar to the procedure employed in Example 2, to thereby construct a plasmid pSV2-1R.

b) Preparation of other DNAs having mutation in the neighborhood of the reactive site

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In order to introduce a mutation in the neighborhood of the reactive site other then 1R, the abovementioned method of Kramer et al. was effected by using tV19-5H3B obtained in Example 2 as a template. Thus mutations of 5R, 26R, 28R, 29R, 30R, 39R, 40R, 46R, 48R, 49R, 50R, 27R, 7R, 34R, 35R, 38R, 9R, 19R, 24R, 2R', 5R' and 6R' were introduced. The amino acid sequence in the neighborhood of the reactive site of each of these AT III mutants is given in Table 3, while the sequences of synthetic oligonucleotides employed for the introduction of the mutations are listed in Tables 1 and 2. Similar to the procedure described in Example 2, a reaction for introducing a mutation was performed in accordance with the manual accompanying the kit and several clones thus formed were collected. Then the nucleotide sequences were determined and thus the clones having the desired mutation introduced thereinto were obtained. From each clone, a DNA fragment of about 1.4 kb was obtained by using Hind III and Bgl II. In the cases of 5R, 26R, 28R, 30R, 27R, 7R, 19R, 24R, 2R', 5R' and 6R', the obtained fragments were replaced with a mouse DHFR gene in pSV2-dhfr in the same manner as those described in Example 2 and Example 3 a) to thereby respectively give plasmids pSV2-5R, pSV2-26R, pSV2-28R, pSV2-30R, pSV2-27R, pSV2-7R, pSV2-19R, pSV2-24R, pSV2-2R', pSV2-5R' and pSV2-6R'. In the cases of 39R, 40R, 46R, 48R, 49R, 50R, 34R, 35R and 38R, on the other hand, each of the DNA fragments was replaced with a part of an NKAF gene in a plasmid pK4K which will be described hereinbelow to thereby respectively give plasmids pK4K-39R, pK4K-40R, pK4K-46R, pK4K-49R, pK4K-50R, pK4K-34R, pK4K-35R and pK4K-38R. In the cases of 29R and 9R, DNA fragments of about 1.4 kb were isolated again from plasmids pSV2-29R and pSV2-9R by using Hind III and BgI II and plasmids pK4K-29R and pK4K-9R were constructed by the same method as those described above.

Example 4

Preparation of heparin binding site-mutated DNA

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Among mutations at the heparin binding site, the mutations of 1G, 2G and 8G were introduced in accordance with the method of Kramer et al. by using tv19-5H3B obtained in Example 2 as a template. The sequences of synthetic oligonucleotides employed therein are given in Table 2. The clones having the

desired mutation introduced thereinto were referred to as 1Gmut, 2Gmut and 8Gmut respectively. From these clones, DNA fragments of about 1.4 kb were excised by using Hind III and Bgl II and treated by the same method as the one employed in the cases of the mutations at the reactive site. Thus plasmids pSV2-1G, pSV2-2G and pSV2-8G were obtained. Further, a DNA fragment of about 730 bp obtained by cleaving pSV2-1G with Hind III and Sac I was inserted into M13Iv19 cleaved with the same enzymes to thereby give tv19-1GN.

The mutations of 1F, 2F, 3F and 7G were introduced in the same manner by using tv19-ATN obtained in Example 2 as a template. The M13 clones having the desired mutation introduced thereinto were referred to as 1Fmut, 2Fmut, 3Fmut and 7Gmut, respectively.

The mutation of 9G was introduced in accordance with the method of Vandeyar et al. with the use of mp19-ATN as a template. The practical operation was performed in accordance with the manual accompanying a kit (T7-GEN In Vitro Mutagenesis System available from USB). First, 1 µg of mp19-ATN single-stranded DNA and 2 pmol of a synthetic oligonucleotide AT9G, the 5'-end of which had been substituted with phosphate with T4 polynucleotide kinase, were heated at 65 °C in 40 mM Tris-HCI pH 7.5 - 20 mM MgCl₂ - 50 mM NaCl for 5 minutes and then slowly cooled to room temperature. To this reaction mixture (10 µl) were added 2 µl of 10 X Synthesis mix (100 mM Tris-HCl pH 7.5 - 20 mM DTT - 5 mM dATP - 5 mM dGTP - 5 mM dTTP - 5 mM 5-methyl-dCTP - 10 mM ATP), 2.5 U of T7 DNA polymerase and 5 U of T4 DNA ligase to thereby give a final volume of 20 µl, followed by allowing to stand at 37 °C for 1 hour. Thus an RF DNA, in which the strand having a mutation introduced thereinto had been exclusively methylated, was synthesized. After inactivating the enzyme by heating the reaction mixture at 70 °C for 10 minutes, 5 U portions of restriction enzymes Msp I and Hha I were added and allowed to react at 37 °C for 45 minutes. Thus one of the DNA strands of the double-stranded DNA used as a template which had not been methylated was exclusively nicked with Msp I and the template single-stranded DNA which had not been replicated into the double-stranded one was cleaved with Hha I.

Subsequently, 50 U of exonuclease III was added to the reaction mixture and allowed to react at 37°C for 45 minutes. Then only the nicked template strand was digested and, as a result, the DNA strand having mutation introduced thereinto was concentrated. After ceasing the reaction by heating at 70°C for 10 minutes, the reaction mixture was transfected into an E. coli SDM strain (mcrAB) free from any restriction system specific for methylated DNA by an ordinary method. Several plaques thus obtained were picked up and DNAs were obtained. Then the nucleotide sequences thereof were determined and thus a clone having the desired mutation introduced thereinto was selected. From the clone thus obtained, a DNA fragment of about 730 bp was isolated by using Hind III and Sac I and inserted into pSV2-5H3B which had been cleaved with the same enzymes to thereby eliminate fragments of the same size. Thus pSV2-9G was obtained.

The 12G mutant was obtained by further introducing a mutation by using a synthetic oligonucleotide AT2G (Table 2) with the use of a DNA having the mutation of 1G introduced thereinto as a template. Namely, it was obtained in accordance with the method of Kramer et al. by using tv19-1GN as a template. After confirming that the desired mutation had been introduced, the obtained clone was referred to as 12Gmut.

The 127G mutant was obtained in accordance with the above-mentioned method of Vandeyar et al. by using a single-stranded DNA of 12Gmut as a template and treating with a synthetic oligonucleotide AT7G. After confirming that the desired mutation had been introduced, the obtained clone was referred to as 127Gmut.

45 Example 5

Preparation of DNA having mutations both in the neighborhood of the reactive site and at the heparin binding site

a) Preparation of 1G5R mutant DNA

A DNA of the 1G5R mutant having a combination of a mutation 1G at the heparin binding site with another mutation 5R in the neighborhood of the reactive site was constructed in the following manner.

The RF DNA of 1Gmut obtained in Example 4 was cleaved with Hind III and Sac I and thus a DNA fragment of about 730 bp having a mutation at the heparin binding site was prepared. The pSV2-5R obtained in Example 3 was cleaved with Sac I and BgI II and thus a DNA fragment of about 670 bp having a mutation in the neighborhood of the reactive site was prepared. These DNA fragments were combined together and inserted into pSV2-dhfr from which a mouse DHFR gene had been eliminated by using Hind III

and Bgl II. Thus pSV2-1G5R was constructed. Further, this pSV2-1G5R was cleaved with Hind III and Bgl II and a DNA fragment of about 1.4 kb thus formed was inserted into a plasmid which was obtained by eliminating a part of a NKAF gene in a plasmid pK4K as will be described hereinafter by cleaving the plasmid pK4K with Hind III and BamH I. Thus pK4K-1G5R was constructed. The preparation of these DNA fragments having mutation and the construction of pSV2-1G5R and pK4K-1G5R by combining these mutated DNA fragments were performed in accordance with publicly known methods. E. coli HB101-pK4K-1G5R containing the plasmid pK4K-1G5R has been deposited with Fermentation Research Institute of Agency of Industrial Science and Technology of the Ministry of International Trade and Industry under the accession number of FERM BP-3806, on March 26, 1992.

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b) Preparation of 2G5R mutant DNA

A DNA of the 2G5R mutant having a combination of a mutation 2G at the heparin binding site with another mutation 5R in the neighborhood of the reactive site was constructed in the following manner.

The RF DNA of 2Gmut obtained in Example 4 was cleaved with Hind III and Sac I and thus a DNA fragment of about 730 bp having a mutation at the heparin binding site was prepared. The pSV2-5R obtained in Example 3 was cleaved with Sac I and BgI II and thus a DNA fragment of about 670 bp having a mutation in the neighborhood of the reactive site was prepared. These DNA fragments were combined together and inserted into pSV2-dhfr from which a mouse DHFR gene had been eliminated by using Hind III and BgI II. Thus pSV2-2G5R was constructed. Further, this pSV2-2G5R was cleaved with Hind III and BgI II and a DNA fragment of about 1.4 kb thus formed was inserted into a plasmid which was obtained by eliminating a part of a NKAF gene in a plasmid pK4K as will be described hereinafter by cleaving the plasmid pK4K with Hind III and BamH I. Thus pK4K-2G5R was constructed. E. coli HB101-pK4K-2G5R containing the plasmid pK4K-2G5R has been deposited with Fermentation Research Institute of Agency of Industrial Science and Technology of the Ministry of International Trade and Industry uncler accession number of FERM BP-3807, on March 26, 1992.

c) Preparation of other both site-mutated DNAs

The DNAs each having a mutation at the corresponding site obtained in Examples 3 and 4 were employed. As a DNA fragment having a mutation at the heparin binding site, DNA fragments of about 730 bp obtained by cleaving pSV2-1G, pSV2-2G, pSV2-9G, 1Fmut, 2Fmut, 3Fmut, 7Gmut, 12Gmut and 127 Gmut each with Hind III and Sac I were prepared. Separately, as a DNA fragment having a mutation at the reactive site, DNA fragments of about 670 bp were obtained by cleaving pSV2-1R, pSV2-5R (or pSV2-1G5R) and pSV2-30R each with Sac I and BgI II. Further, pK4K-35R was cleaved with Sac I and Xho II to thereby give a DNA fragment of about 670 bp (In a DNA prepared by inserting an AT III mutant gene with a Hind III-Bgl II fragment into a plasmid wherein a part of an NKAF gene had been eliminated from pK4K by cleaving with Hind III and BarnH I, the Bgl II-cleaved end is connected to the BarnH I-cleaved end. Thus it is impossible to cleave this DNA again with Bgl II. However, this site can be cleaved with Xho II.). These DNA fragments were combined together and then inserted into a plasmid wherein a part of the NKAF gene had been eliminated from pK4K by cleaving with Hind III and BamH I. Thus pK4K-1G30R, pK4K-1G35R, pK4K-2G30R, pK4K-2G35R, pK4K-1F5R, pK4K-2F5R, pK4K-3F5R, pK4K-7G5R, pK4K-7G30R, pK4K-7G35R, pK4K-9G5R, pK4K-9G30R, pK4K-9G35R, pK4K-12G5R, pK4K-12G30R, pK4K-12G35R, pK4K-127G5R, pK4K-127G30R and pK4K-127G35R were constructed. Furthermore, pSV2-1G1R and pSV2-2G1R were constructed in a similar manner by using pSV2-dhfr from which a mouse DHFR gene had been eliminated with the use of Hind III and BgI II.

Example 6

50 Construction of expression vector for animal cells

a) Construction of natural recombinant AT III and 1R expression vector

A plasmid pNK8308 (disclosed in European Patent Publication-A3 No. 357067) containing a cDNA coding for recombinant natural killer cell activating factor (NKAF) was digested with Bgl II and BamH I and electrophoresed on an agarose gel. Thus an NKAF cDNA fragment of about 0.75 kb was isolated. A plasmid pKCR [see O Hare, K. et al., Proc. Natl. Acad. Sci. USA, 78, 1527 (1981)] was digested with BamH I and dephosphorylated with alkaline phosphatase. The vector DNA thus obtained was connected (ligated) to the

NKAF cDNA fragment by adding T4 DNA figase to thereby give pKCRNK (Fig. 1).

A plasmid pUC19 was digested with Pst I, then treated with T4 DNA polymerase by a conventional method to thereby blunt (to thereby be blunt-ended) both of the 3'- and 5'-ends and then ligated, thus giving pUC19Pst1. Subsequently, this pUC19Pst1 was digested with BamH I and dephosphorylated with alkaline phosphatase. The vector DNA thus obtained was ligated with a DNA fragment of about 2.4 kb [containing adenovirus promoter, mouse dihydrofolate reductase (DHFR) gene and SV40 polyA signal], which had been isolated by digesting a plasmid pAdD26SV(A) (no.3) [see Kaufmann, R. and Sharp, P., Mol. Cell. Biol., 2, 1304 (1982)] with BamH I and electrophoresing on an agarose gel, to thereby give pUC19Pst-Ad (Fig. 2). Further, this pUC19Pst Ad was digested with Pst I and blunt-ended with T4 DNA polymerase and then ligated to thereby give pUC19Pst - AsPst -. Then a DNA fragment of about 2.9 kb containing a tetracycline-resistant gene, which had been isolated by digesting pAdD26SV(A) (no. 3) with BamH I, dephosphorylating and electrophoresing on an agarose gel, was ligated with another DNA tragment of about 2.4 kb containing adenovirus promoter, mouse DHFR gene and SV40 polyA signal, which had been isolated by digesting pUC19Pst AdPst with BamH I and electrophoresing on an agarose gel, to thereby give pAdPst" (Fig. 3). Then the pAdPst" was digested with EcoR I and blunt-ended by treating with a DNA polymerase I Klenow fragment. Subsequently, it was digested with Pst I and blunt-ended with T4 DNA polymerase. Then Aat II linker was added thereto and ligated therewith and the obtained product was digested with Aat II and electrophoresed on an agarose gel. Thus a DNA fragment of about 2.7 kb containing adenovirus promoter, mouse DHFR gene and SV40 polyA signal was obtained. This DNA fragment was ligated with a DNA obtained by digesting pKCRNK with Aat II and dephosphorylating to thereby give pKCRNKAd (Fig. 4).

The plasmid pSV2-5H3B obtained in Example 2 was digested with Hind III and Bgl II and a DNA fragment of about 1.4 kb containing AT III cDNA was isolated. This fragment was ligated with a vector DNA obtained by digesting a plasmid pIC19R [see Marsh, J.L. et. al., Gene, 32, 481 (1984)] with Hind III and Bgl II to thereby give pIC19R5H3B. Next, this pIC19R5H3B was digested with BamH I and Bgl II and a DNA fragment of about 1.4 kb containing 5H3B cDNA was isolated. This DNA fragment was ligated with a vector DNA obtained by digesting pKCR with BamH I and dephosphorylating to thereby give pKCR5H3B (Fig. 5).

pKCRNKAd was digested with Aat II and a DNA fragment of about 2.7 kb containing adenovirus promoter, mouse DHFR gene and SV40 polyA signal was isolated. This DNA fragment was ligated with a vector DNA obtained by digesting pKCR5H3B with Aat II and dephosphorylating to thereby give pKCR5H3BAd (Fig. 6). The pKCR5H3BAd was used in order to express a natural recombinant AT III in animal cells as will be described in Example 7.

Similarly, by the use of pSV2-1R obtained in Example 3 a) as the starting material, pKCR1RAd was obtained. The pKCR1RAd was used in order to express a mutant 1R in animal cells as will be described in 35 Example 7.

b) Construction of expression vectors of various mutants in animal cells

pKCR5H3BAd was digested with EcoR I and then self-ligated. Thus pKCRAdEco wherein SV40 promoter, a part of the NKAF gene and a part of rabbit \$\beta\$-globin gene had been eliminated was selected. The pKCRAdEco was digested with BamH I, blunt-ended with a DNA polymerase I Klenow fragment and then ligated to thereby give pKCRAdEcoB⁻. Subsequently, the pKCRAdEcoB⁻ was digested with Hind III, blunt-ended with a DNA polymerase I Klenow fragment and then ligated to thereby give pKCRAdEcoB⁻H⁻- (Fig. 7).

pKCRNKAd was digested with Hind III and BamH I and a DNA fragment of about 0.4 kb containing a part of the NKAF gene was isolated. Then it was ligated with a vector DNA obtained by digesting pIC19R with Hind III and BamH I to thereby give pIC19RNKK. The pIC19RNKK was digested with BgI II and BamH I and a DNA fragment of 0.4 kb containing a part of the NKAF gene was isolated. This DNA fragment was ligated with a vector DNA obtained by digesting pKCR with BamH I and dephosphorylating to thereby give pKNK (Fig. 8).

pKNK was partially digested with EcoR I and a DNA fragment of about 1.5 kb containing SV40 promoter, a part of NKAF gene and a part of rabbit β -globin gene was isolated. Then this DNA fragment was ligated with a vector DNA obtained by digesting pKCRAdEcoB⁺H⁻ with EcoR I and dephosphorylating to thereby give pK4K (Fig. 9).

As Fig. 9 shows, pK4K contains the promoter of an early gene of SV40, the replication initiation region of SV40, a part of the NKAF gene, a part of the rabbit β -globin gene (splicing and polyA signal), the polyA signal of the early gene of SV40, the major late gene promoter and the 5' splice signal of type II adenovirus, rabbit immunoglobulin 3' splice signal, mouse DHFR gene, the polyA signal of the early gene of SV40, the

replication initiation region of pBR322 and a β -lactamase gene originating in pBR322 (Amp γ) and the dhfr was connected on the downstream side of the major late gene promoter of adenovirus and a part of the NKAF gene was connected on the downstream side of the promoter of the early gene of SV40.

An expression vector in animal cells can be constructed by inserting an AT III mutant gene into a site remaining after excising a part of the NKAF gene of pK4K with Hind III and BamH I. In practice, expression vectors of the mutants 1G5R and 2G5R were prepared by using pSV2-1G5R and pSV2-2G5R respectively and pK4K by the above-mentioned method as shown in Example 5 a) and b). These vectors were referred to as pK4K-1G5R and pK4K-2G5R. As described in Example 3 b) and Example 5 c), expression vectors of other mutants were similarly constructed by using pK4K.

c) Construction of expression vectors of mutants 5R and 7R

The plasmid pSV2-5R obtained in Example 3 b) was digested with Hind III and BgI II and thus a DNA fragment of about 1.4 kb containing a 5R gene was isolated. This DNA fragment was ligated with a vector DNA obtained by digesting pKNK with Hind III and BamH I to thereby eliminate a part of the NKAF gene, thus giving pKNK5R. The pKNK5R was digested with EcoR I and a DNA fragment of about 1.5 kb containing the promoter of the early gene of SV40, a 5R gene and a part of the rabbit \$\beta\$-globin gene was isolated. This DNA fragment was ligated with a vector DNA obtained by digesting pKCRAdEcoB⁺H⁻ with EcoR I and dephosphorylating to thereby give pKCR5RAd (Fig. 10). Similarly, pKCR7RAd was obtained by using pSV2-7R obtained in Example 3 b).

Example 7

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Expression of AT III mutant by animal cells

a) Expression by CHO cell

CHO cells [dhfr-deficient strain, see Urlaub, G. and Chasin, L.A., Proc. Natl. Acad. Sci. USA, 77, 4216 (1980)] were inoculated in an incubation flask at a ratio of 7 x 10^s cells/5 ml/the flask of 25 cm². On the next day, 3 µg of the plasmid pKCR1RAd obtained in Example 6 a) was transfected by the calcium phosphate method with the use of a CellPhect (a kit manufactured by Pharmacia). As a medium, one obtained by adding fetal calf serum to a 1:1 mixture (a DF medium) of Ham F12 medium with Dulbecco's modified Eagle medium in such a manner that the obtained medium contained 10% of the fetal calf serum, was used. After 3 days, the cells were trypsinized and diluted with a selection medium (DF medium free from hypoxanthine and thymidine + 10% dialyzed fetal calf serum). Then 1 ml portions of the cells contained in one incubation flask (25 cm²) were pipetted into each of wells of four 24-well plates for incubation and the incubation was continued in the selection medium while replacing the medium with a fresh one at intervals of 3 to 4 days. Cells surviving under these conditions were those transformed by the mouse DHFR gene. After approximately 2 weeks, the colonies thus formed were dispersed by trypsinizing in wells and a fresh medium was added, followed by incubating for additional 3 to 4 days. Then the culture broth was exchanged and the amount of 1R contained in the culture supernatant was determined by the EIA method on the next day. Each clone showing an expression yield of about several ten ng/ml/day or more was transinoculated into a selection medium containing 50 nM of methotrexate (MTX) and incubated for 2 to 3 weeks. Further, the MTX concentration was successively elevated to 100 nM, 400 nM and 1000 nM and the incubation was continued in the same manner. Among clones growing at the MTX concentration of 1000 nM, those showing high expression yields were cloned by the limiting dilution method with the use of a 96well plate. In the state of confluent growth, a clone 110-6, which was a typical example of those thus obtained, secreted about 10 µg/ml/day of 1R into the culture supernatant at 0.3 ml of the medium/cm2. Similarly, CHO cells capable of expressing a natural recombinant AT III were obtained by using pKCR5H3BAd obtained in Example 6 a).

- b) Expression of various mutants by BHK cell
- i) Use of pSV2 vectors

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The plasmids shown in Fxamples 3 and 5, which were constructed by replacing the mouse DHFR gene in a plasmid pSV2-dhfr by an AT III mutant DNA, can be used for expressing various mutants by transfecting into animal colls together with pSV2-dhfr (cotransfection).

BHK cells [tk-ts13 strain, see Waechter, D.E. and Baserga, R., Proc. Natl. Acad. Sci. USA, 79, 1106 (1982)] were inoculated in an incubation flask at a ratio of 5 × 10⁵ cells/5 ml/the flask of 25 cm². On the next day, 7 μg of a plasmid pSV2-28R having a gene of a mutant 28R shown in Example 3 b) introduced thereinto was transfected into the BHK cells together with 3.5 μg of pSV2-dhli by the calcium phosphate method with the use of CellPhect. As a medium, one obtained by adding fetal call serum to Dulbecco's modified Eagle medium in such a manner that the obtained medium contained 5% of the fetal call serum, was used. After 3 days, the cells were trypsinized and subcultured into a 75 cm² incubation flask with a medium containing 200 nM of MTX. After incubating for 10 days while replacing the medium with a fresh one at intervals of 2 to 3 days, the cells were subcultured into a 175 cm² incubation flask with a medium containing 1000 nM of MTX. After incubating for additional 10 days while replacing the medium with a fresh one at intervals of 2 to 3 days, a cell strain showing a high expression yield was cloned by the limiting dilution method with the use of a 96-well plate. A clone #4 thus obtained secreted about 0.7 μg/ml/day of 28R into the culture supernatant at 0.3 ml of the medium/cm² in a state of confluent growth. Regarding the plasmids containing other mutant DNAs which were constructed with pSV2 and described in Examples 3 and 5, expression cells could be obtained by the same method as the one described above.

ii) Use of other vectors

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BHK cells (tk-ts13 strain) were inoculated in an incubation flask at a ratio of 3 × 10⁵ cells/5 ml/the flask of 25 cm². On the next day, 3 μg of a plasmid pK4K-2G5R having a gene of a mutant 2G5R obtained in Example 5 b) introduced thereinto was transfected into the BHK cells by the calcium phosphate method with the use of CellPhect. As a medium, one obtained by adding fetal calf serum to Dulbecco's modified Eagle medium in such a manner that the obtained medium contained 5% of the fetal calf serum, was used. After 2 days, the cells were trypsinized and diluted with a medium containing 250 nM of MTX. The cells in one 25 cm² incubation flask were pipetted into wells of twelve 24-well plates for incubation. Then the incubation was continued while replacing the medium with a fresh one at intervals of 3 to 4 days. After 12 days, the colonies thus formed were dispersed by trypsinizing in the wells and the medium was added. After incubating for additional 6 days, the culture broth was exchanged. On the next day, the amount of each mutant contained in the culture supernatant was measured by the EIA method and a cell strain showing a high expression yield was cloned. A clone 6-5 thus obtained secreted about 16 μg/ml/day of 2G5R into the culture supernatant at 0.3 ml of the medium/cm² in a state of confluent growth.

Regarding pKCR1RAd, pKCR5RAd and pKCR7RAd described in Example 6 and the plasmids containing other mutant DNAs described in Examples 3 and 5, which were constructed by using pK4K, expression cells were obtained in a similar manner. Further, cells capable of expressing the natural recombinant AT III could be obtained by the same method with the use of pKCR5H3BAd shown in Example 6.

Some of these expression cells were transinoculated into a medium containing 1000 nM of MTX and further incubated. Some of clones incubated in the medium containing 1000 nM of MTX, which showed high expression yields, were cloned by the limiting dilution method with the use of a 96-well plate.

The expression yields of typical examples of the clones thus obtained were shown in Table 10.

Table 10

	Expre	ssion of AT III mutant by BHK cell	
Mutant	Clone	Amount of secretion into medium (µg/ml)	MTX concn. (nM)
natural recombinant AT III	F242	15-20	1000
1R	5-41	25-30	1000
5R	D153	13-15	1000
7R	3-153	20-25	1000
1G5R	11-1	10	1000
6R'	5-21	15	1000
30R	6-18	19	1000
2G5R	6-5	16	250
25R	4-2	20	250
35R	42-5	22	250
29R	22-8	17	250
2G30R	16	19	250
7G5R	1	12	250

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The amount of secretion into the medium was expressed in the mutant concentration 24 hours after replacing the medium in a state of confluent growth of cells (the amount of medium was 0.3 ml/cm²).

Example 8

Incubation of mutant expression cells and purification of mutant

The AT III mutant expression cells obtained in Example 7 were incubated in a roller bottle (1750 cm²). As a medium, Dulbecco's modified Eagle medium containing 5% of fetal calf serum and MTX (final concentration being 250 nM or 1000 nM) was used. The cells were inoculated into 300 ml of the medium and incubated at 37 °C. From 3 to 4 days after the initiation of the incubation, the medium was replaced by the same amount of a fresh one everyday and the culture supernatants were combined.

The AT III mutants were purified by affinity chromatography with the use of an antibody column wherein anti AT III monoclonal antibody was bound to a support. Namely, the above-mentioned culture supernatant was charged into an antibody column which had been equilibrated with 50 mM Tris-HCl buffer pH 7.5 - 0.5 M NaCl. After washing with the same buffer, it was eluted with 0.2 M glycine-HCl buffer (pH 2.5). The eluted fractions were immediately neutralized with 1/2 times by volume as much 1 M Tris-HCl (pH 8.0). The fractions thus obtained were dialyzed against Dulbecco's PBS (-), ultrafiltrated and then used in the subsequent test. In the cases of some mutants, the eluted fractions from the antibody column was ultrafiltrated, charged into Sepharcryl S-200 and eluted with Dulbecco's PBS (-) (gel-filtration). The active fraction thus obtained was concentrated and then used in the subsequent test.

During the process of incubation and purification, each AT III mutant was determined by the EIA method with the use of anti AT III antibody.

The natural recombinant AT III employed as a control was also incubated and purified by the same 45 method.

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SEQUENCE LISTING

(EPO)
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27

e-merers :

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	(2) INFORMATION FOR SEQ ID NO: 1:
5	(i) SEQUENCE CHARACTERISTICS:
	(A) LENGTH: 1395 base pairs
	(B) TYPE: nucleic acid
10	(C) STRANDEDNESS: double
	(D) TOPOLOGY: linear
15	(ii) MOLECULE TYPE: cDNA
	(vi) ORIGINAL SOURCE:
20	(A) ORGANISM: Homo sapiens
	(ix) FEATURE:
	(A) NAME/KEY: CDS
25	(B) LOCATION: 11395
	(ix) FEATURE:
30	(A) NAME/KEY: sig_ peptide
	(B) LOCATION: 196
	(ix) FEATURE:
35	(A) NAME/KEY: mat_ peptide
	(B) LOCATION: 971395
40	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:
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45	Met Tyr Ser Asn Val lle Gly Thr Val Thr Ser Gly Lys Arg Lys Val
	-32 -30 -25 -20
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15				20)				25					30)			
	GAT	GAG	GGC	TCA	GAA	CAA	AAG	ATC	CCG	GAG	GCC	ACC	CAAC	CGG	CG	r GT	C 240	
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	11	e Se	er G]	u Le	u Va	l Tyi	Gly	r Ala	a Lys	s Le	u Gl:	n Pr	o Le	u As	p Pl	he L	ys	
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SEQUENCE LISTING

_	(1) GENERAL INFORMATION:
5	(i) APPLICANT: (A) NAME: Eisai Co., Ltd. (B) STREET: 6-10, Koishikawa 4-chome, Bunkyo-ku (C) CITY: Tokyo (E) COUNTRY: Japan (F) POSTAL CODE (21P): 112
	(ii) TITLE OF INVENTION: Human Antithrombin III Mutants
	(iii) NUMBER OF SEQUENCES: 81
75	(iv) COMPUTER READABLE FORM: (A) MEDIUM TYPE: Floppy disk (E) COMPUTER: IBM PC compatible (C) OPERATING SYSTEM: PC-DOS/MS-DOS (D) SOFTWARE: Patentin Release #1.0, Version #1.25 (EPO)
20	(vi) PRIOR APPLICATION DATA: (A) APPLICATION NUMBER: JP 90488/92 (B) FILING DATE: 10-Apr-1992 (A) APPLICATION NUMBER: JP 31855/93 (B) FILING DATE: 22-Feb-1993
25	(2) INFORMATION FOR SEQ ID NO: 1:
3 <i>0</i>	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 1395 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: double (D) TOPOLOGY: linear (ii) MOLECULE TYPE: cDNA
35	<pre>(vi) ORIGINAL SOURCE: (A) ORGANISM: Homo sapiens (ix) FEATURE: (A) NAME/KEY: CDS (B) LOCATION: 11355</pre>
40	(ix) FEATURE: (A) NAME/KEY: sig_peptide (B) LOCATION: 196
45	(ix) FEATURE: (A) NAME/KEY: mat_peptide (B) LOCATION: 971395
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:
50	ATG TAT TCC AAT GTG ATA GGA ACT GTA ACC TCT GGA AAA AGG AAG GTT 48 Met Tyr Ser Asn Val lle Gly Thr Val Thr Ser Gly Lys Arg Lys Val -32 -30 -25

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5	Нī	C GGG S Gly l	G AGG	C CC	CTC Val	GAC Asp	ATC	TGC Cys	AC/	A GCC Ala	Ly	S CC	G CG	GAG G Asi	C AT	CCC Pro	144
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	GAC Asp	ATG Met	GGC	CTT Leu 340	GTC Val	GAT Asp	CTG Leu	TTC Phe	AGC Ser 345	CCT Pro	GAA Glu	AAG Lys	TCC Ser	AAA Lys 350	CTC Leu	CCA Pro	1152
25	GGT Gly	ATT Ile	GTT Val 355	GCA Ala	GAA Glu	GGC Gly	CGA Arg	GAT Asp 360	GAC Asp	CTC Leu	TAT Tyr	GTC Val	TCA Ser 365	GAT Asp	GCA Ala	TTC Phe	1200
30	CAT His	AAG Lys 370	GCA Ala	TTT Phe	CTT Leu	GAG Glu	GTA Val 375	AAC Asn	G⊅A Glu	GAA Glu	GI'y	AGT Ser 380	GAA Glu	GCA Ala	GCT Ala	GCA Ala	1248
	AGT Ser 385	ACC Thr	GCT Ala	GTT Val	GTG Val	ATT Ile 390	GCT Ala	ej À eec	CGT Arg	TCG Ser	CTA Leu 395	AAC Asn	CCC Pro	AAC Asn	AGG Arg	GTG Val 400	1296
35	ACT Thr	TTC Phe	AAG Lys	Ala	AAC Asn 405	AGG Arg	CCT Pro	TTC Phe	Leu	GTT Val 410	TTT Phe	ATA Ile	AGA Arg	GAA Glu	GTT Val 415	CCT Pro	1344
40	CTG Leu	AAC Asn	Thr	ATT Ile 420	ATC Ile	TTC : Phe !	ATG Met	Gly.	AGA Arg ' 425	GTA Val	GCC . Ala .	≟AC ≛sn	Pro	TGT Cys 430	GTT Val	A-G Lys	1392
	TAA				•												1395

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:(A) LENGTH: 464 amino acids(B) TYPE: amino acid(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

55

45

		ix)	.) SI	EQUEI	NCE !	DESC	RIPT	ION:	SEQ	ID	NO:	2:				
5	Me1 - 31	Tyr 2	-30	Ası O	n Va	1 11	= Gly	y Thi -25	r Va. 5	l Th	r Se	r G1	y Ly:		J Ly	s Val
J	Ту	- Leu	Lev	ı Sei	. Lei	ı Lev	Le:	:1e	• G1	y Pho	≥ Trị	o Ası		s Val	1 Th	c Cys
10	His I	s Gly	Sei	Pro	Va]	l Asi) Ile	Cys	፣ ፓክ	r Ala		s Pro	Arq) Ası) Ile	e Pro
70	Met	Asn.	Pro	Met 20	Cys	s Ile	туг	Arg	Sei 25		Glu	Lys	s Lys	30		c Glu
15	Asp	Glu	G1 y 3 5	Ser	Glu	Glr	Lys	11e 40		Glu	ı Ala	Thi	Asr 45		Arç	y Val
15	Trp	Glu 50	Leu	Ser	Lys	: Ala	Asn 55	Se <i>r</i>	Arç	Phe	Ala	Thi 60		Phe	туг	Gln
20	His 65	Leu	Ala	Asp	Ser	Lys 70	Asn	Asp	Asn	Asp	Asn 75		: Fhe	: Leu	Sez	Pro 80
20	Leu	Ser	Ile	Ser	Thr 85	Ala	Phe	Ala	Met	Thr 90		Leu	Gly	Ala	Cys 95	Asn
or	Asp	Thr	Leu	Gln 100	Gln	Leu	Met	Glu	Val 105	Phe	Lys	Phe	Asp	Thr 110		Ser
25	Glu	Lys	Thr 115	Ser	Asp	Gln	Ile	His 120		Phe	Phe	Ala	Lys 125		Asn	Cys
20	Arg	Leu 130	Tyr	Arg	Lys	Ala	Asn 135		Ser	Ser	Lys	Leu 140		Ser	Ala	Asn
30		Leu	Phe	Gly	Asp	Lys 150	Ser	Leu	Thr	Phe	Asn 155		Thr	Tyr	Gln	Asp 160
25	Ile	Ser	Glu	Leu	Val 165	Tyr	Gly	Ala	Lys	Leu 170	Gln	Pro	Leu	Asp	Phe 175	
35	Glu	Asn	Ala	Glu 180	Gln	Ser	Arg	Ala	Ala 185	lle	Asn	Lys	Trp	Val 190	Ser	Asn
40		Thr	195					200					205			
		Leu 210					215					220				
45	223	Lys				230					235					240
45		Ala .			245					250					255	•
· .		Phe .		260					265					270		
50	Pro	Phe :	Lys 275	Gly .	Asp	Asp	lle	Thr 280	Met	Val	Leu		Leu 285	Pro	Lys	Pro

	C7	u Ly 29		er Le	u Al	a Ly		al G 45	le L	7.E (51 u	Leu	?hr 300		o 63	ט עב) Leu	
5	G1 30		u Ti	r Le	u As		lu L 10	eu G	jn C	<u>)</u> u }	1e t	Мет 315	Leu	Va <u>l</u>	Ve	l Hi	s Met 320	
	Pr	o Ar	g Ph	e Ar	g 11 32		u A	sp G	ly P		Ser S30	Leu	Lys	G) u	G1:	n Le 33	ս Gln 5	
10	As	p He	τ 61	y Le 34	u Va O] As	p Le	eu Pi		er P 45	,:0	Glu	ŗ7.2	Ser	Ly:		u Pro	
	GJ ;	y 31	e Va 35		æ 61	n ej	y Ar		sp As 50	sp L	eυ	Tyr	Va]	Ser 365		s XI	a Phe	
	His	s Ly:		a Phe	e Le	u G]	υ Va 37		sr G]	lu G	lu		Ser 380	Glu	Ala	a Ala	Ala	
75	Se:	r Th:	r Ala	a Val	l Va	11 29	e Al O	a G)	y Ar	g S	er :	Leu 395	Asn	Pro	Asr	Arç	Val 400	
	Thi	Phe	e Lys	s <u>#1</u> s	40:		g Pr	o Ph	e Le		al i	Phe	17e	Arg	Glu	Val 415	Pro	
20	Leu	Asr	Th 1	11e 420		÷ Phe	e Me	t Gl	y Ar 42		al A	ula 2	Asn	Pro	Cys 430		Lys	
•	(2)	INF	FORN	TI OH	FOF	S SEC) ID	ио:	3:								•	
25			(A) L	ENCE ENGT	ዝ: 4	64 a	nime										
			(D) T	YPE: OPOL	OGY:	lir	near										
30					LE T CE D		•			ID.	NO:	3:						
	Met -32	Tyr	Ser -30	Asn	Val	Ile	Gly	Th:		Th	r 5	er G		-20	Arg	Lys	Val	
35 ·	Tyr	Leu -15	Leu	Ser	Leu	Leu	Leu -10		617	, Ph	e T.		sp (:)'s	Val	Thr	C).a	
	His 1	вīу	Ser	Fro	Val 5	Asp	Ile	Cys	Thr	Ala 10		ys P	ro A	irg .	Asp	11e 15	Pro	
40	Met	Asn	Pro	Met 20	Cys	lle	Tyr	Arg	Ser 25		o 61	lu L	ys L	ys)	41a 30	Thr	Glu	
	Asp	Glu	Gly 35	Ser	Glu	Gl n	Lys	11e 40	Pro	Gli	ιAl	la Ti	nr A	.sn 3 45	Arg	Arg	Val	
15	Trp	G) ս 50	Leu	Ser	Lys	Ala	Asn 55	Ser	Arg	Phe	: A1		or T 50	hr E	Pbe '	туг	Gln	
	His 65	Leu	Æla	Asp	Ser	Lys 70	Asn	Asp	Asn	Asp		n 11	e P	he I	eυ.	Ser	Pro EO	
5 0	Leu	Ser	lle	Ser	Thr 85	Ala	Phe	Ala	Met	Thr 90	L).	s Le	υ G	ly A	la (Cy's 2 95	Asn	

	A.	sp T	hr Le	ຍນ G1: 10	n G1 O	n Le	u Me	t G1	υ Va	11 Ph	ie Ly	s Ph	e As	p Th 11		e Ser
5	G.	lu L	ys Th	r Se. .5	r As	p Gl	n Il	e Hi 12	s P} 0	e Ph	e Ph	e Al	a Ly 12		u As	n Cys
	A.	rg Le 1:	eu Ty 30	r Ar	g Ly	s Al	a As:	n Ly 5	s Se	r Se	r Ly	s Le 14	u Vai 0	l Se	r Al	a Asn
10	• •					150	,				25	5				n Asp 160
					10.	,				17	0				175	
15				100	,				18	5				190)	Asn
			13.	J				200)				205)		e Asn
20		21	U	r Val			215)				220)	•		
				t Lys		230					235	•				240
25				o Gly	243	1				250)				255	
				7 Tyr 260					265	•				270		
30			273					280					285			
	٠	230	,	Leu			295					300				
35	505			Leu		310					315					320
				Arg	323					330					335	
40				140 340					345					350		
	٠.		رر	Ala Phe				360					365			
45		3.0		Val '			3/5					380				
				Ala /		390					395					400
50				Ile I	103					410	•				415	
				420				- x y	425	vaı ,	wrg ,	usn .		30 Sys	/al :	Lys

(2) INFORMATION FOR SEQ ID NO: 4:

s			(1)	(A)	LENC TYPE	CE CH STH: C: an OLOGY	464 nino	ami: acid	nc ad							
		(i	i) M	OLEC	ULE	TYPE	: pr	Ctei	in							
		(×.	i) S	EQUE	NCE	DESC	RI PT	: 4O I	SEC) ID	NO:	4:				
10	Ме -3	t Ty. 2	r Se -3	r As O	n Va	1 11	e Gl	y Tr. -2		1 Th	r Se	r Gl	у L y -2		g Ly	's Va
	Ty.	r Lei	ı Let	υ Se	r Le	u Le	u Le -1		e G]	y Ph	e Tr	p Ası		s Va	l Th	r Cys
15	Hi	s Gly 1	Se	r Pr	o Va	1 As ₁ 5	p Il	e Cy	s Th		a Ly	s Pro	o Ar	g As	11 1	e Pro
20	Me	t Asn	Pro	: Me: 21	c Cys	s Ile	е Ту	r Ar	g Se. 21		o Cj	u Lys	s Ly:	s A.1;		r Glu
23	Asp	o Glu	G1,	Se:	r Glu	ı Glr	a Lys	; Il		o G11	u Ali	∍ Thr	Ası 45		g Ar	g Val
25	Trp	61u 50	Leu	Sei	Lys	s Ala	Asr 55	Se:	r Arq	Phe	÷ Ala	Thr 60		: Phe	ту:	r Gln
	His 65	Leu	Ala	Asp	Ser	: Lys 70		Asp) Asn) Asp	75		Phe	Lev	Se:	Pro 80
30	Leu	Ser	Ile	Ser	Thr 85	Ala	Phe	A.la	Met	Thr 90		Leu	GT %	Ala	Су: 95	Asn
	. Asp	Thr	Leu	Gl n 100	Gln	Leu	Met	Glu	Val 105		Lys	Phe	Asp	Thr 110		Ser
35	Glu	Lys	Thr 115	Ser	Asp	Gln	Ile	His 120	Phe	Phe	Phe	Ala	Lys 125	Leu	Asn	Cys
	Arg	Leu 130	Tyr	Arg	Lys	Ala	A.s.n 135	Lys	Seı	Ser	Lys	Leu 140	Vēl	Ser	Ala	Asn
40	Arg 145	Leu	Phe	Gly	Asp	Lys 150	Ser	Leu	Thr	Phe	Asn 155	Glu	Thr	Tyr	Gln	Asp 160
	Ile	Ser	Glu	Leu	Val 165	Tyr	Gly	Ala	Lys	Leu 170	Gln	Pro	Leu	Asp	Phe 175	Lys
45	Glu	Asn	Ala	Glu 180	Gln	Ser	Arg	Ala	Ala 185	Ile	Asn	Lys	Тгр	Val 190	Ser	Asn
	Lys	Thr	Glu 195	G] À	Arg	Ile	Thr	Asp 200	Val	Ile	Pro		Glu 205	Ala	lle	Asn
50	Glu	Leu 210	The	Val	Leu	Val	Leu 215	Val	Asn	Thr	lle.	Туг 220	Phe	Lys	61 y	Leu ,

	T:	rp Ly 25	ys Se.	r Lys	?he	Se: 230	Pro	Glu	Asn	The	Arc 235	Lys ,	G) (J Let	ı Phe	e Ty 24€
5	Ly	ys Al	la Asp	• €1 ₃	Glu 245	Ser	Cys	Ser	: Ala	Ser 250	Met	. Met	туг	: Glr	Glv 255	
	Γ7	ys Ph	e Arç	7yr 260	Arg	Arg	. Val	Ala	Glu 265	Gly	Thr	Gln	Val	Leu 270		ı Lev
10	Pz	o Ph	e Lys 275	Gly	Asp	Asp	Ile	Thr 280	Met	Val	Leu	lle	Leu 285		Lys	Pro
	G1	u Ly 29	s Ser O	Leu	Ala	Lys	Val 295	Glu	Lys	Glu	Leu	Thr 300	Pro	Glu	Val	Let
75	G1 30	n Gl 5	u Trp	Leu	Asp	Glu 310	Leu	Glu	Glu	Met	Met 315	Leu	Val	Val	His	Met 320
	Pr	o Ar	g Phe	Arg	Ile 325	Glu	Asp	Gly	?he	Ser 330	Leu	Lys	Glu	Gln	Leu 335	
20	As	p Me	t Gly	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	Glu	Lys	Ser	Lys 350	Leu	Pro
	Gl	y Ile	e Val 355	Ala	Gl u	Gly	Arg	Asp 360	Asp	Leu	Tyr	Val	Ser 365	Asp	Ala	Phe
25	Hi	s Lys 370	s Ala	Phe	Leu	Glu	Val 375	Asn	Glu	Glu	Gly	Ser 380	Glu	Ala	Ala	Ala
	Se:	r Thi	: Ala	Val	Val	Ile 390	Phe	Pro	Arg	Ser	Leu 395	Asn	Pro	Asn	Arg	Val 400
30	Th	Phe	Lys	Ala	Asn 405	Arg	Pro	Phe	Leu	∨al 410	Phe	Ile	Arg	Glu	Val 415	Pro
	Lev	ı Asn	Thr	Ile 420	Ile	Phe	Met	Gly	Arg 425	Val.	Ala	Asn	Pro	Cys 430	Val	lys
35	(2)	INF	(B	EQUE	NCE (NGTH: PE: a	CHAR : 46 amin	ACTE 4 am 5 ac:	RIST ino . id	: ICS: acid:	s						
10) MOL													
	Met		SEQ										· • • • • •	N== 1	·	
5			-30 Leu :					23				-	-20			
						_	10					- 5				
o	_		Ser I		,					10					15	
	Met	Asn	Pro M	let C 20	ys I	le T	yr A	rg S	er P 25	ro G	lu L	ys L	ys A	la T 30	hr G	lu

	As	p G1	ս Gl 3		r Glu	. G3:	n Ly		e Pr O	c 63	u Ai	a Tì.		n Ar 5	g At	g Val
s	Tri	p G1 5		u Se	r Lys	: A.)	a As 5		r Ar	g Ph	e Ala	a Th é		: Ph	e T;	r Gla
5	Hi:		u ንኒ	a Asi	S S E I	7(1):		n Asj	p As	n As	p Asi 7:		e Ph	e Le	บ Se	r Pro 80
	Let	Se:	r 11:	e Sei	Thr E5		Phe	e Ala	a Me	t Th S		s Le	u 61	y Ala	а С;; 9	s Asr. 5
10	Asp	Thi	r Lei	01r 100		Leu) Met	: Glı	va: 10:		e Lys	: Phe	e Asj	p Th:		e Ser
	G) u	Lys	115		Asp	Gln	Ile	His 120		≞ Phe	e Phe	: Ala	ь Ly: 12:		ı Ası	n Cys
15	Arg	Leu 130		Arg	Lys	≱l a	Asn 135		Sei	r Sei	Lys	le:		l Sei	: Ala	a Asn
	Arg 145	Leu	Phe	Gly	Asp	Lys 150		Leu	Thr	? Phe	155		The	туг	Glr	160
20	∑1e	Ser	Glu	Leu	Val 165	Tyr	СŢĀ	Ala	Lys	Leu 170		Pro	Leu	Asp	Phe 175	Lys
	Glυ	Asn	Ala	Glu 180	Gln	Ser	Arg	Ala	Ala 185		Asn	Lys	Trp	Val 190		Asn
25	Lys	Thr	61u 195		Arg	Ile	Thr	Asp 200	Val	Ile	Pro	Ser	Glu 205		Ile	Asn
	Glu	Leu 210	Thr	Val	Leu	Val	Leu 215	Val	Asn	Thr	Ile	Tyr 220	Phe	Lys	G] y	Leu
30	Trp 225	Lys	Ser	Lys	Phe	Ser 230	Pro	Glu	Asn	Thr	Arg 235	Lys	Glu	Leu	Phe	Tyr 240
	Lys	Ala	Asp	Gly	Glu 245	Ser	Cys	Ser	Ala	Ser 250	Met	Met	Tyr	Gln	Glu 255	СŢŸ
35	Lys	Phe	Arg	Tyr 260	Arg	Arg	Val	Ala	Gl u 265	Gly	Thr	Gln	Val	Leu 270	Glu	Leu
	Pro	Phe	Lys 275	Gly	Asp.	Asp	Ile	Thr 280	Met	Val	Leu	lle	Leu 285	Pro	Lys	Pro
40	Glu	Lys 290	Ser	Leu	Ala		Val 295	Glu	Lys	Glu		Thr 300	Pro	Glu	Val	Leu
	305					310					315			•		320
45					Ile (325					330					335	•
	Asp :			340					345					350		
50	Gly :	Ile	Val . 355	Ala	Glu (51 y 2		Asp . 360	4.sp	Leu	Tyr \		Ser 365	Asp.	Ala	Phe

	ні	s Ly 37	s Al 0	a Pin	ie re	u 61	u Va 37	1 As 5	n GJ	u Gl	u G1	y Se 38		u Al	a Al	a Ala
5	Se 38	r Th 5	r Al	a Va	l Va	1 Il	e Il O	e P:	o Àr	g Se	r Le		n Pr	o As	n Ar	g Va. 400
	Th	r Ph	e Ly	s Al	a As 40	n Are 5	g Pr	o Ph	e Le	บ Va: 41		e Il	e Ar	g Gl	u Val 41	
10	Le	u As:	n Th	r Il 42	e Il O	e Phe	e Me	€ G1	y Ar 42		l Ali	a As	n Pr	o Cy: 430		l Lys
	. (2) IN	FORM	ATIO	N FO	R SE() ID	NO:	6:							
15				(A) : (B) :	UENC: LENG' TYPE TOPO:	TH: 4 : ami	164 a	amin bio								
		(1)	i) Mo	DLEC	ULE :	TYPE:	pro	cteir	ה							
20	:	(xi	L) SI	EQUE!	NCE I	DESCR	RIPT	EON:	SEQ	ID N	10: E	:				
	Met -32	Ty:	-30	Ası	n Val	l Ile	e G13	/ Thi	r Val	l Thr	Ser	Gly	/ Lys		, Lys	Val
25	Ту	-15	Leu	ı Se	r Lei	J Leu	Let -10	1 1 l e	e G1 y	y Phe	Trp	A.s.p -5	cy:	s Val	Thr	Cys
	His 1	G1 y	/ Ser	Pro	Va)		Ile	: Cys	Th	Ala 10		Pro	Arç) Asp	11e	
30	Met	Asn	Pro	Met 20	Cys	Ile	Tyr	Arg	Ser 25		Glu	Lys	Lys	30		Glu
	Asp	Glu	Gly 35	Ser	: Glu	Gln	Lys	11e 40		Glu	Ala	Thr	Asn 45		Arg	Val
35	Trp	61 u 50	Leu	Ser	Lys	Ala	Asn 55	Ser	Arg	Phe	Ala	Thr 60		Phe	туг	Gln
	His 65	Leu	Ala	Asp	Ser	Lys 70	Asn	Asp	Asn	Asp	Asn 75	Ile	Phe	Leu	Ser	Pro 80
40	Leu	Ser	Ile	Ser	Th: 85	Ala	Phe	Ala	Met	Thr 90	Lys	Leu	Gly	Ala	Cys 95	Asn
				100				-	105	٠.				110		
45	Glu	Lys	Thr 115	Ser	Asp	Gĺn	Ile	His 120	Phe	Phe	Phe	Ala	Lys 125	Leu	Asn	Cys
•	Arg	Leu 130	Tyr	Arg	Lys	Ala	Asn 135	Lys	Ser	Ser	Lys	Leu 140	Val	Ser	Ala	Asn
	Arg 145	Leu	Phe	ej À	Asp	Lys 150	Ser	Leu	Thr	Phe	Asn 155	Glu	Thr	Tyr	Gln	Asp 160
50	Ile	Ser	Glu	Leu	Val 165	Tyr	G1 y	Ala	Lys	Leu 170	Gln	Pro	Leu		Phe	Lys

	Gin	ı Ası	n Ala	18) (3)		, 5e	r Ar	g Al	= Al 18		e As:	n Ly:	s Tr	p Va. 19		r Asn
5	Lys	Th	r Glu 193		y Arg	11	e Th	r As 20		1 11	e Pro	s Se	r Gl (ā 11·	e Asn
	G) t	210 210	The	: Val	Leu	va.	l Let 215		l Ası	n Thi	r Ile	± Ty:		Ł)	e 61;	y Leu
10	Trp 225	Lys	Ser	Lys	Phe	Se: 230		G]ı	ı Asr	Thi	235		Glu	le:	Phe	7yr 240
	Lys	Ala	Asp	Gly	Glu 245		Cys	Se <i>t</i>	: Ala	Ser 250		. Met	Туг	Glr	61 255	G1 y
15	Lys	Phe	Arg	Туг 260		Arg	yal	⊁.1 a	61u 265		Thr	Gln	Val	Leu 270		Leu
	Pro	Phe	Lys 275	Gly	Asp	Asp	Ile	Thr 280		Val	Leu	lle	Leu 285	Pro	Lys	Pro
20	G1 u	Lys 290	Ser	Leu	Æla	Lys	Val 295	G1 u	Lys	Glu	Lev	Thr 300	Pro	Glu	Val	Leu
	Gln 305	Glu	Trp	Leu	Asp	Glu 310		Glu	Glu	Met	Мет 315	Leu	Vel	Val	His	Meτ 320
25	Pro	Arg	Phe	Arg	11e 325	Glu	Asp	G] À	P'ne	Ser 330	Leu	Lys	.G1 u	Gln	Leu 335	Gln
	Asp	Met	Gly	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	Glu	Lys	Ser	Lys 350	Leu	Pro
30	Gly	Ile	Val 355	Ala	G1 บ	Gly	Arg	Asp 360	Asp	Leu	Tyr	Val	Ser 365	Asp	Ala	Phe
	His	Lys 370	Ala	Phe	Leu	Glu	Val 375	Asn	Glu	Glu	Gl y	Ser 380	Glu	Ala	Ala	Ala
35	Ser 385	Thr	Ala	Val	Val	Ile 390	Gly	Pro	Arg	Ser	Leu 395	Asn	Pro	Asn	Arg	Val 400
	Thr	Phe	Lys	Ala	Asn . 405	Arg	Pro	Phe	Leu	Val 410	Phe	Ile	Arg		Val 415	Pro
. 40	Leu .	Asn	Thr	Ile 420	lle	Phe	Met		Arg 425	Val.	Ala.	Asn		Cys 430	Val	Lys
	(2)	INFO	PMAT:	HOI:	FOR S	S E Q	א פו	0: 7	:							•
45			(A)	LE:	NCE (NGTH: PE: a POLOG	4 6 mi.n	4 am:	ino a id		5						
50		(ii)	MOLE	CUL	TYP	E: j	DIOLE	ein								
	1	(x:i)	SEQU	ENCE	DES	CRI!	PTION	∛: SE	EO II) NO:	7:					

	Ме - 3	т Ту 2	r Se -3	r As	n Va	1 11	e Gi	у Т ћ -2	r Va 5	1 Tt.	r Se	r Gl	y Ly -2		g Ly	s Va
5	Ту	r Le	u Le 5	u Se	r Le	u Le	u Le -1	ս Il 0	e Gl	y Ph	e Tr	p As -		s Va	l Th	r Cy:
	Hi	s Gl l	y Se	r Pr	o Va	l Asy 5	o Il	е Су	s Th	r Al		s Pr	o Ar	g As	p Il l	
10	. Me	t As	n Pr	o Met 20	c Cy	s Ile	⇒ Ty:	r Ar	g Se 2	r Pre 5	o G1	u Ly	s Ly	s Al 3		r Gl
	As	p Gl	u Gl 3	y Sei 5	r Gl	ı Gl	ı Lys	5 Il 4	e Pr	o G1	ı Ala	a Th	r As:		g Ar	g Val
15	Tr	P G1:	u Le	u Sei	Lys	s Ala	Ası 55	n Se.	r Ar	g Phe	≥ Ala	Th:		r Ph	е Ту	c Glr
	Hi:	s Lei 5	u Ala	a Asp	Se:	: Lys 70	Asr	n Ası	p Ası	n Asp	75 75		e Phe	e Le	u Sei	Pro 80
20	Let	u Sei	r Ile	e Ser	Th:	Ala	Ph∈	2 Ala	a Met	Thr 90		. Le	. GJ	/ Ala	a Cys 95	
	Asp	Thi	. Lei	100	Glr	Leu	Met	: Gl	Va] 109		. Lys	Phe	e Asr	Th:		: Ser
25			113					120)				125	5		
		130	,	Arg			135	•				140)			
30	143	•		: Gly		150					155	'				160
				Leu	165					170					175	
35				Glu 180					185					190		
			132					200					205			
40		210		Val			215					220				
	223			Lys		230					235					240
45				Gly	245					250					255	_
				791 260					265					270		
50			273	Gly				280					285			
	Glu	Lys 290	Ser	Leu .	Ala	Lys :	Val 295	Glu	Lys	Glu	Leu	Thr 300	Pro	G] u	Val	Leu

	305 305		u Trj) Lei	ı Azş	310 ن 311		o 61	u Glu) Me	315		u Va	i Va.	l Hi	s Met 320
5	Pro	s Arq	g Phe	e Arg	325		بر Asy	G)	y Phe	: Se:		e Ly.	s Gl	u Gli	n Le: 33.	u 61 n 5
	.Asp	. Met	c 617	y Let 340		l Asp	b Lei	ı Ph	≥ Ser 345		s Glu	Ly:	s Se	25 (u Prc
10	6 3 7	, Ile	e Val 355		61 v	. CJ)	/ Arg	360 360		. Leu	Tyr	Va.	365		> 7.1 :	a Phe
·	Ľis	370		Phe	Leu	Glu	vel 375		ı Glu	Glu	Gly	Se:) Ala	. Ala	a Ala
75	Ser 385		: Ala	Val	Val	1)e 390		Pro	Arg	Ser	Leu 395		Pro	Asn	Arç	y Val 400
	Thr	Phe	: Lys	Ala	Asn 405	-) Pro	Phe	· Leu	Val 410		Ile	: Arg	61 u	Val 415	Pro
20	Leu	Asn	Thr	11e 420		Phe	Met	Gly	Arg 425		Ala	Asn	Pro	Cys 430		. Lys
	(2)	INF	AMSO.	TI ON	FOR	SEQ	ID	NO:	8:							
25		•	(.	SEQU Al L B) T D) T	ENGT: YPE :	H: 4	64 a:	mino cid								
				LECU:			-									
30	Met -32	Tyr		QUEN Asn					_				Lys -20	Arg	Lys	Val
35	Tyr	Leu -15	Leu	Ser	Lev	Leu	Leu -10	Ile	Gly	Phe	Тгр	Asp -5	Cys	Val	Thr	Cys
	His 1	Gly	Ser	Pro	Val 5	Asp	Ile	Суѕ	Thr	Ala 10	Lys	Pro	Arg	Asp	Ile 15	Pro
4D	Met	Asn	Pro	Met 20	Cys	Ile	Tyr	A.r g	Ser 25	Pro	Glu	Lys	Ly's	Ala 30	Thr	Glu
	Asp		Gly 25	Ser	Glu	Gln	Lys	11e 40	Pro	Gľυ	Ala	Thr	Asn 45	Arg	Arg	Val
45	Trp	Glu 50	Leu	Ser	Ly's	<i>F</i> .1 a	Asn 55	Ser	Arg	Phe	Ala	Thr 60	Thr	Phe	туг	Gln
45	His 65	Leu	Ala	Asp	Ser	L չ։s 70	Asn	Asp	Asn.	Asp	Asn 75	Ile	Phe	Leu	Ser	Pro 80
	Leu	Ser	lle	Ser	Thr 85	Ala	Phe	Ala	Met	Thr 90	Lys	Leu	Gly	Ala	Суs 95	Asn
50	Asp	Thr	Lev	Gln 100	Gln	Leu	Met	Glu	Val 105	Phe	Lys	Phe		Thr 110	Ile	Ser

	Glu Lys	Thr Ser	Asp (Gln Ile	: His !	Phe Phe	Phe Ala	Lys Le	u Asn Cys
					120			125	
5	250			133	1		140		r Ala Asn
	Arg Leu 145	Phe Gly	Asp I	Lys Ser 150	Leu ?	Thr Phe	Asn Glu 155	Thr Ty	r Gln Asp 160
10	Ile Ser	Glu Leu	Val T 165	yr Gly	Ala L	ys Leu 170	Gln Pro	Leu Asj	Phe Lys
	Glu Asn	Ala Glu 180	Gln S	er Arg	Ala A	la Ile . 85	Asn Lys	Trp Val	l Ser Asn
75	Lys Thr	Glu Gly 195	Arg I	le Thr	Asp V 200	al Ile	Pro Ser	Glu Ala 205	lle Asn
	Glu Leu 210	Thr Val	Leu V	al Leu 215	Val A	sn Thr :	le Tyr 220	Phe Lys	Gly Leu
20	Trp Lys 225	Ser Lys	Phe S	er Pro 30	Glu A	sn Thr A	Arg Lys 235	Glu Leu	Phe Tyr 240
	Lys Ala	Asp Gly	Glu Se 245	er Cys	Ser Al	la Ser N 250	let Met	Tyr Gln	Glu Gly 255
25	Lys Phe	Arg Tyr 260	Arg A	rg Val	Ala G1 26	lս Gly T 55	hr Gln	Val Leu 270	Glu Leu
	Pro Phe	Lys Gly 275	Asp As	sp Ile	Thr Me 280	t Val L	eu Ile	Leu Pro 285	Lys Pro
30	Glu Lys : 290	Ser Leu	Ala Ly	s Val (Glu Ly	s Glu L	eu Thr ! 300	Pro Glu	Val Leu
	Gln Glu 7 305	rp Leu .	Asp Gl 31	u Leu (O	Glu Gl	u Met M	et Leu \ 15	/al Val	His Met 320
35	Pro Arg E	he Arg	Ile G1 325	u Asp (Sly Ph	e Ser Le 330	eu Lys G	lu Gln	
	Asp Met G	ly Leu \ 340	/al As _i	p Leu P	he Se	r Pro G] 5	lu Lys S	er Lys 350	Leu Pro
40	Gly Ile V	al Ala G 55	lu Gly	y Arg A 3	sp Asi 60	Leu Ty		er Asp 65	Ala Phe
• • .	His Lys A	la Phe I	eu Glu	val A 375	sn Glu	ı Glu Gl	y Ser G 380	lu Ala /	Ala Ala
45	Ser Thr A	la Val V	al Ile 390	Trp P.	ro Arg	Ser Le 39	ບ Asn Pi 5	ro Asn)	Arg Val 400
	Thr Phe Ly	ys Ala A 4	sn Arg 05	Pro Pi	he Leu	Val Pho 410	e Ile Ar		
50	Leu Asn Th	11e I: 420	le Phe	Met Gl	ly Arg 425	Val Ala	a Asn Pr	O Cys \ 430	al Lys

	(2) INFORMATION FOR SEQ ID NO: 9:
s	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 464 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear
	(ii) MOLECULE TYPE: protein
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:
10	Met Tyr Ser Asn Val Ile Gly Thr Val Thr Ser Gly Lys Arg Lys Val -32 -30 -25 -20
	Tyr Leu Leu Ser Leu Leu Leu Ile Gly Phe Trp Asp Cys Val Thr Cys -15 -10 -5
75	His Gly Ser Pro Val Asp Ile Cys Thr Ala Lys Pro Arg Asp Ile Pro 1 5 10
	Met Asn Pro Met Cys lle Tyr Arg Ser Fro Glu Lys Lys Ala Thr Glu 20 25 30
20	Asp Glu Gly Ser Glu Gln Lys Ile Pro Glu Ala Thr Asn Arg Arg Val 35 40 45
	Trp Glu Leu Ser Lys Ala Asn Ser Arg Phe Ala Thr Thr Phe Tyr Gln 50 . 55 60
25	His Leu Ala Asp Ser Lys Asn Asp Asn Asp Asn Ile Phe Leu Ser Pro 65 70 75 80
	Leu Ser Ile Ser Thr Ala Phe Ala Met Thr Lys Leu Gly Ala Cys Asn 85 90 95
30	Asp Thr Leu Gln Gln Leu Met Glu Val Phe Lys Phe Asp Thr Ile Ser 100 105 110
	Glu Lys Thr Ser Asp Gln Ile His Phe Phe Phe Ala Lys Leu Asn Cys 115 120 125
35	Arg Leu Tyr Arg Lys Ala Asn Lys Ser Ser Lys Leu Val Ser Ala Asn . 130 135 140
	Arg Leu Phe Gly Asp Lys Ser Leu Thr Phe Asn Glu Thr Tyr Gln Asp 145 150 155 160
40	Ile Ser Glu Leu Val Tyr Gly Ala Lys Leu Gln Pro Leu Asp Phe Lys 165 170 175
	Glu Asn Ala Glu Gln Ser Arg Ala Ala Ile Asn Lys Trp Val Ser Asn 180 185 190
45	Lys Thr Glu Gly Arg Ile Thr Asp Val Ile Pro Ser Glu Ala Ile Asn 195 200 . 205
	Glu Leu Thr Val Leu Val Leu Val Asn Thr Ile Tyr Phe Lys Gly Leu 210 215 220
50	Trp Lys Ser Lys Phe Ser Pro Glu Asn Thr Arg Lys Glu Leu Phe Tyr 225 230 235
	Lys Ala Asp Gly Glu Ser Cys Ser Ala Ser Met Met Tyr Gln Glu Gly

					2 -	45					250					25	55
5	Ly	s Ph	e Ar	g Ty 26	/r A: 50	g A:	eg Va	al A	la G 2	1 u 65	Gly	Thr	Gl	n Va.	1 Le 27		u Le
	Pr	o Ph	e Ly 27	s G <u>1</u> 5	y As	sp As	p Il	ie Ti 21	hr M 30	let	Vaì	Leu	114	28		o Ly	s Pr
10	Gli	u Ly 29	s Se O	r Le	u Al	a Ly	's Va 29	1 G1 15	lu L	ys I	Glu	Leu	Th:	Pro	G 1	u Va	l Le
	305 305	n Gl 5	u Trj	p Le	u A.s	p G1 31	u Le 0	u Gl	u G	lu I	Met	Met 315	Leu	ı Va]	. Va	1 Hi	5 Me 32
15	Pro	Ar	g Pho	e Ar	g Il 32	e Gl 5	u As	p Gl	y P	he :	Ser 330	Leu	Lys	Glu	Gl	n Le 33	
	Asp) Me	t Gly	7 Le 34	u Va O	l As	p Le	u Ph	e S	er B 45	Pro	G1 u	Lys	Ser	Ly:		u Pr
20	GT A	, 11	e Val	Al.	a Gl	u Gl	y Ar	g As 36	р А. 0	sp I	beu	Tyr	Val	Ser 365		Al.	a Ph
	His	370	s Ala	Pho	e Le	u Gl	u Va 37	1 As 5	n G]	lu G	5lu	Gly	Ser 380	Glu	Ala	a Ala	a Ala
25	Ser 385	Thi	Ala	Va)	l Va	1 II 39	e Va O	l Pr	o A.	g S	Ser	Leu 395	Asn	Pro	Asr	Ar	y Val 400
			Lys		40:	•				4	10					415	5
30	Leu	Asn	Thr	11e	: Ile	e Ph€	e Met	Gl;	y Ar 42	g V 5	al.	Ala	Asn	Pro	C y s 4 3 0		Lys
	(2)	INF	ORMA	TION	FOF	R SEÇ	Q ID	NO:	10:								
35			(i) (i) (i)	SEQU A) L B) T	ENCE ENGT YPE:	CHA H: 4 ami OGY:	RACT 64 a	ERIS mino cid	TIC	5: ids						•	
) MOI														
80	Met -32		Ser -30					Thr	Va]				51 y		Arg	Lys	Va <u>l</u>
	Tyr	Leu -15		Ser	Leu	Leu	Leu -10	-25 Ile		, Ph	e T	rp A	\sp	-20 Cys	Val	Thr	Cys
5	His		Ser	Pro	Val 5	Asp		Суз	Thr	: Al	a L	ys P	-5 _.	Arg.	Asp		Pro
	Met 2	Asn	Pro	Met 20	Cys	Ile	Tyr	Arg	Ser 25	Pr		lu L	ys]	Lys i	Ala 30	15 Thr	Glu
0	Asp (Glu	Gly :	Ser	Glu	Gln	Lys	11e 40	Pro	Gl	u Al	la T	hr A	Asn <i>)</i> 45		Arg	Val

	Tr	p G1 5		u Sei	Lys	: Al	a As 5		r Ar	g Ph	e Al		r Th	= Ph	е Ту	r Gl
s	Hi:		u ኡኒል	≞ Asp	Se:	ւ Լ չ։ 7		n As	p As	n As	р As 7		e Ph	e Le	u Sè	r Pr
	Per	ı Se:	r Ile	: Ser	Thr 85		a Ph	e Al	a Me	t Th		s Le	u 61	y Al	ā C) [.]	-
10	Asp	Th	r Leu	61n 100		Let	u Met	c Gl	u Va 10		e Ly:	s Pho	e As	р Th 11	r 11	e Se.
	Glu	Lys	Thr 115		Asp	Glr	ı Ile	# His 120		e Pho	e Phe	e Ala	12		u As:	יצ ר
15	Arg	130		Arg	Lys	Ala	135		s 5e:	r Sei	r Lys	let 140		l Se.	r Ala	s Ası
	Arg 145		Phe	G1 y	Asp	Lys 150		Let	Thi	r Phe	2 Asn 155		Th:	г Ту:	r Glr	160
20	Ile	Ser	Glu	Leu	Val 165	Tyr	: Gly	Ala	Lys	170		Pro	Lev) Asp	Phe 175	
	Glu	A.sn	Ala	Glu 180	Gln	Ser	Arg	Ala	Ala 185		: Asn	Lys	Trp	7 Val	Sez)	: Asn
?5			195					200	٠				205	•	: Ile	
		210					215					220			Gly	
0	. 225		•	•		230					235				Phe	240
	•				245					250					Glu 255	
5				260					265					270		
			275			•		280					285		Lys	
o		290					295					300			Val	
	305					310					315				His	320
5					325					330					Leu 335	
				340					345					350	Leu	
0			355					360					365		Ala Ala	
	3	370			J-4 (. · u	375	ve u	GT U	o i u		ser 380	OT II	∵⊤a	WT 9	ΨT9

	5 d	er Ti 35	ir Al	la Va	al Va	al 13	le Le 90	eu Pi	10 A.	19 Se	er Le	eu As 95	sn Pi	:0 As	n Az	g Val 400
5	T)	nr Ph	e L	ys Al	la As 40	sn A; 05	rg Pa	o Pł	ne Le	eu Va 41	1 Ph	e Il	le Ar	:g G)	u Va 41	l Pro 5
	Le	eu As	n Tì	r Il 42	e I1 20	e Pl	ne Me	t Gl	y Ai 43	g Va 25	1 A1	a As	n Pr	о Су 43		l Lys
10	{2	!) IN	FORM	OITA	N FO	R SE	Q 10	CN C	11:							
15				(A) (B) (D)	UENC LENG TYPE TOPO	TH: : am LOGY	464 ino : li	amin acid near	o ac	S: ids						
					ULE											
	W -				NCE											
20	- 5	_	- 3	·				-2	5				-2	0		val
	Ty.	r Lei	u Lei 5	u Se.	r Le	u Le	u Le	u Il 0	e Gl	y Phe	e Tr	Ası -	р Су: 5	s Val	l Thi	Cys
25	Hi:	s Gly l	y Se.	r Pro	o Val	l As	p Il	e Cy	s Th	r Ala	a Ly:	S Pro	o Are	g Asp) Ile 15	Pro
	Me	Ası	n Pro	2 Met	t Cys	5 Il	е Ту	r Arq	Se. 2:	r Pro	G) G)	Ly:	s Lys	Ala 30		Glu
30	Asp	Glu	Gl y 35	y Ser	c Glu	ı Glı	Lys	11e	e Pro	o Glu	Ala	Thi	: Asr 45		Arg	Val
	Trp	50 50	Leu)	Ser	Lys	: Ala	Asn 55	Ser	Arç	, Phe	: Ala	Thr 60		Phe	Туг	Gln
35	His 65	Leu	Ala	Asp	Ser	Lys 70	Asn)	Asp	Asn	Asp	Asn 75		Phe	Leu	Ser	Pro 80
	Leu	Ser	Ile	Ser	Thr 85	Ala	Phe	Ala	Met	Thr 90	Lys	Leu	Gly	Ala	Cys 95	Asn
. 40	Asp	Thr	Leu	Gln 100	Gln	Leu	Met	Glu	Val 105	Phe	Lys	Phe	Asp	Thr 110	Ile	Ser
	Glu ·	Lys	Thr 1:15	Ser	Asp	Gln	Ile	His 120	Phe	Phe	Phe	Ala	Lys 125	Leu	Asn	_. Cys
45	Arg	Leu 130	Tyr	Arg	Lys	Ala	Asn 135	Lys	Ser	Ser	Lys	Leu 140	Val	Ser	Ala	Asn
40	Arg 145	Leu	Phe	Gly	Asp	Lys 150	Ser	Leu	Thr	Phe	Asn 155	Glu	Thr	Туг	Gln	Asp 160
	Ile	Ser	Glu	Leu	Val 165	Tyr	Gly	Ala	Lys	Leu 170	Gln	Pro	Leu	Asp	Phe 175	Lys
50	Glu	Asn	Ala	Glu 180	Gln	Ser	Arg	Ala	Ala 185	Ile	Asn	Lys	Trp	Val 190	Ser	Asn

	Lys	Thr	Gl u 195	-	Arg	lle	The	Asp 200		116	Pro	Ser	61 u 205		11€	A.E.rs
5	Glu	Leu 210		Val	Leu	Val	Leu 215		Asn	Thr	11e	220 17:1		Ly's	G] Y	Leu
	Trp 225	_	Ser	ГЛZ	Phe	Ser 230		Glu	Asn	Thr	Arg 235	Lys	G) u	Leu	Phe	Tyr 240
10	Lys	λla	Asp	G1 y	Gl u 245	Ser	Cys	Ser	λla	Ser 250	Меτ	Het	Tyr	Gln	Gl u 255	Gly
	Lys	Phe	Arg	Туг 260	Arg	Arg	Val	Ala	G1 u 265	G] Y	Thr	Gln	Val	Leu 270	Glu	Leu
15	Pro	Phe	Lys 275	Gly	Asp	Asp	Ile	Thr 280	Met	Vāl	Leu	Ile	Leu 285	Pro	Lys	Pro
	Glu	Lys 290	Ser	Leu	Ыa	Lys	Val 295	G1 u	Lys	G1 u	Leu	Thr 300	Pro	Glu	Val	Lev
20	Gln 305	Glu	Trp	Leu	Asp	Glu 310	Leu	Glu	Glu	Meť	Met 315	Leu	Val	Val	His	Met 320
	Pro	Arg	Phe	Arg	11e 325	G] u	Asp	6 53	Phe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	Gln
25	Asp	Met	Gly	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	Glu	Lys	Ser	Lys 350	Leu	Pro
	Gly	Ile	Val 355	Ala	Glu	€ĵ À	Arg	Asp 360	Asp	Leu	Tyr	Val	Ser 365	Asp	Ala	Phe
30		Lys 370					375				·	380				
	Ser 385					390					395					400
35		?he			405					410					415	
	Leu	Asn '		11e 420	Ile	Phe	Met	_	Arg 425	Val .	Ala .	Asn :		Cys 430	Val	Lys
40	(2)	INFO:	RMAT	ION	FOR :	SEQ :	ום או	D: 1	2:							
		(:	(A.) LE	NCE (NGTH PE: 4	: 46	4 am.	ino		5						
<i>4</i> 5		(ii)	(D) то	POLO	3Y: :	linea	a r								
		(xi)				•			EQ 11	: טא כ	12:					
50	Met '		Ser) -30	Asn Y	/al]	le (Thr \ 25	/al 7	Thr S	Ser G		ys . 20	rg I	Lys \	/al

	Tyr	Leu Le -15	u Ser	Leu	Leu	Leu -10	l Ile	Gly	?'ne	Trp	Asp -5	Cys	Val	Thr	C7.2
5		5ly Se							10					15	
		lsn Pro						23					30		
10		lu Gly 35										45			
		lu Leu 50									60				
15		eu Ala								15					80
		er Ile							30					95	
20	Asp T							103					110		
	Glu L _}	s Thr 115	Ser &	Asp G	Sln :	Ile	His 1 120	Phe E	he P	he A	la L 1	ys 1 25	eu A	sn C	ys
25	Arg Le 13	u Tyr O	Arg I	ys A	la A	Asn 1 135	Lys s	Ser S	er L	ys Le le	eu V. 10	al s	er A	la A	sn
	Arg Le 145	u Phe	Gly A	sp L 1	ys S 50	er i	Ceu 1	hr P	he As	sn G] 55	iu Ti	hr T	yr G		sp 60
30	Ile Se	r Glu	Leu V 1	аl Т 65	yr G	ly A	la L	ys L	eu Gl 70	n Pr	o Le	au A		ne Ly 75	/ S
	Glu Ası	Ala	Glu G 180	ln Se	er A	rg A	la A 1	la I: 85	le As	n Ly	s Tr	р V.	al Se	er As	n
35	Lys Thi					_	00				20	5			
-	Glu Leu 210	Thr \	'al Le	u Va	1 Le 21	eu Va 15	al As	n Th	r Ile	P Ty:	Pho	e Ly	s Gl	y Le	u
40	Trp Lys 225			-	-				233	1				240)
70	Lys Ala							2.0	,				255	ı Gly	,
	Lys Phe						20.	,				27(u Glu	ı Leu	
45	Pro Phe	Lys G) 275	y Asp	Asp) 11	e Th.	r Met 0	: Val	Leu	Ile	Leu 285	Pro	Lys	Pro	
	Glu Lys 290									300					
50	Gln Glu ' 305	Trp Le	u Asp	Glu 310	Leu	Glu	Glu	Met	Met 315	Leu	Val	Va l	His	Met 320	

	Pr	o As	g Ph	ė Ar	g 11 32		u As	p G)		Ser 1 :30	Leu :	ŗŅs	Glu	61	n Le 33	
5	As.	p Me	t Gl	y Le 34	u Ya O) As	p Le	u Pi		er F 45	ro (31u !	Lys	Ser	25		u Ps
	G)	y I1	e Va. 35		a G1	и 6 1	y Ar	9 As 36		sp L	ev J	,ř.t /	/al	Ser 365		p 7.1	a Ph
10	Hi	s Ly 37		a Ph	e be	u Gl	υ Va 37		n Gl	Lu G	lu G		Ser SBO	Glυ	A. 1	a Al	a Al
	Se 38		r Aja	e Va	l Val	1 Le		e Pr	ıA o	:g S		eu A 95	.sn	Pro	٨sı	n As	9 Va. 40
15	T'n.	r Ph	e Lys	s Ala	a Asr 405		g Pr	o Ph	e Le		al P 10	he I	le	Arg	G11	Va: 41	-
·	Let	u Ası	n Thi	11: 420	≘ Ile	: Phe	e Mei	c G1	y Ar 42		el A	la A	n2.	Pro	C y s 4 3 0		l Ly:
20	(2)	INE	- -1450-	10 IT.	FOF	. SEQ) ID	NO:	13:								
			(A) 1	ENCE ENGT YPE:	H: 4	164 a	mino									
25		(11			OFOL)								
		(xi) SE	QUEN	CE D	ESCR	IPTI	ON:	SEQ	ID	ю:	13:					
30	Met -32		Ser 30		Val	īle	Gly	Thr		l Th	r Sē	r Gl		Lys -20	Arg	Lys	Val
·	Tyr	16u -15	Leu	Ser	Leu	Lev	Leu -10	Ile	Gly	r Ph	e Tr		sp C	Cys	Val	The	Cys
35	His 1		Ser	Ь́го	Val 5	Asp	Ile	Cys	Thi	A).		s Pr	:o A	rg	Asp	11e 15	Pro
	Met	Asn	Pro	Met 20	C7.2	Ile	Tyr	Arg	Ser 25		o Gl	n Fà	'5 L	ys .	Ala 30	Thr	Glu
10			35		Glu			4 C						45			
		50			Lys		55					6	O				•
15	65				Ser	70					7.	5					03
•					Thr 85					90)					95	
io				100	Gln				105					1	10		
·=	Glu	Lys	Thr 115	Ser	Asp	Gln	J)e	His 120	Phe	Phe	Phe	Ala		ys 1 25	eu.	Asn	Cys

	Arg Leu Tyr Arg Lys Ala Asn Lys Ser Ser Lys Leu Val Ser Ala Asn 130 135 140
5	Arg Leu Phe Gly Asp Lys Ser Leu Thr Phe Asn Glu Thr Tyr Gln Asp 145 150 155 160
	Ile Ser Glu Leu Val Tyr Gly Ala Lys Leu Gln Pro Leu Asp Phe Lys 165 170 175
10	Glu Asn Ala Glu Gln Ser Arg Ala Ala Ile Asn Lys Trp Val Ser Asn 180 185 190
	Lys Thr Glu Gly Arg Ile Thr Asp Val Ile Pro Ser Glu Ala Ile Asn 195 200 205
15	Glu Leu Thr Val Leu Val Leu Val Asn Thr Ile Tyr Phe Lys Gly Leu 210 215 220
-	Trp Lys Ser Lys Phe Ser Pro Glu Asn Thr Arg Lys Glu Leu Phe Tyr 230 235 240
20	Lys Ala Asp Gly Glu Ser Cys Ser Ala Ser Met Met Tyr Gln Glu Gly 245 250 255
	Lys Phe Arg Tyr Arg Arg Val Ala Glu Gly Thr Gln Val Leu Glu Leu 260 265 270
25	Pro Phe Lys Gly Asp Asp Ile Thr Met Val Leu Ile Leu Pro Lys Pro 275 280 285
	Glu Lys Ser Leu Ala Lys Val Glu Lys Glu Leu Thr Pro Glu Val Leu 290 295 300
30	Gln Glu Trp Leu Asp Glu Leu Glu Glu Met Met Leu Val Val His Met 305 310 315 320
	Pro Arg Phe Arg Ile Glu Asp Gly Phe Ser Leu Lys Glu Gln Leu Gln 325 330 335
35	Asp Met Gly Leu Val Asp Leu Phe Ser Pro Glu Lys Ser Lys Leu Pro 340 345 . 350
	Gly Ile Val Ala Glu Gly Arg Asp Asp Leu Tyr Val Ser Asp Ala Phe 355 360 365
40	His Lys Ala Phe Leu Glu Val Asn Glu Glu Gly Ser Glu Ala Ala Ala 370 380
	Ser Thr Ala Val Val Ala Tyr Pro Arg Ser Leu Asn Pro Asn Arg Val 385 390 395 400
45	Thr Phe Lys Ala Asn Arg Pro Phe Leu Val Phe Ile Arg Glu Val Pro 405 410 415
	Leu Asn Thr Ile Ile Phe Met Gly Arg Val Ala Asn Pro Cys Val Lys 420 430

(2) INFORMATION FOR SEQ ID NO: 14:

55

s			1	(A) [(B) [JENC! LEHG! LYPE: LOPO!	FH: : am	464 . ino .	amin bioe	ç. ≥ <i>C</i>							
					ICE I		•			ID 1	10: :	14:				
70	Met - 32	-	Ser -30		. Val	. 110	e G) 3	7 Th:		l Thi	: 5e :	c Gly	y Lys		Lys	: Val
	Tyr	Leu -15		Ser	: Leu	Leu	-10		e G) ;	y Phe	Trp	Asp 		: Val	Thz	Cys
15	His 1	_	Ser	Pro	Val 5		, lle	: Cys	Th:	Ala 10	_	Pro	Arç	Asp	11e	Pro
	Иet	⊁.sn	Pro	Met 20		116	Туг	Arç	Se :		Glu	Lys	Lys	71a 30		Glu
20			35					40	•				45	'	_	Val
		50					55					60				Gln
25	65			_		70		•			75					Pro 80
				_	Thr 85					90			_		95	
30				100	Gln				305		_		-	110		
			115		Asp			120					125			_
35		130			Lys		135	_			_	140				
	145				Asp Val	150					155			-		160
4O					165 Gln					170				-	175	_
				180	Arg				185				•	190		
45			195		Leu			200					205			
		210	•		Phe		215					220				
50	225					230					235					240
					245		-			250			-		255	•

	Lys Phe Arg Tyr Arg Arg Val Ala Glu Gly Thr Gln Val Leu Glu Leu 260 265 270
5	Pro Phe Lys Gly Asp Asp Ile Thr Met Val Leu Ile Leu Pro Lys Pro 275 280 285
	Glu Lys Ser Leu Ala Lys Val Glu Lys Glu Leu Thr Pro Glu Val Leu 290 295 300
10	Gin Glu Trp Leu Asp Glu Leu Giu Glu Met Met Leu Vai Val His Met 305 310 315 320
	Pro Arg Phe Arg Ile Glu Asp Gly Phe Ser Leu Lys Glu Gln Leu Gln 325 330 335
15	Asp Met Gly Leu Val Asp Leu Phe Ser Pro Glu Lys Ser Lys Leu Pro 340 345 350
	Gly Ile Val Ala Glu Gly Arg Asp Asp Leu Tyr Val Ser Asp Ala Phe 355 360 365
20	His Lys Ala Phe Leu Glu Val Asn Glu Glu Gly Ser Glu Ala Ala 370 375 380
	Ser Thr Ala Val Val Ala Trp Pro Arg Ser Leu Asn Pro Asn Arg Val 385 390 395 400
25	Thr Phe Lys Ala Asn Arg Pro Phe Leu Val Phe Ile Arg Giu Val Pro 405 410 415 Leu Asn Thr Ile Ile Phe Met Gly Arg Val Ala Asn Pro Cys Val Lys
	420 425 430
30	(2) INFORMATION FOR SEQ ID NO: 15:
	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 464 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear
35	(ii) MOLECULE TYPE: protein
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15: Met Tyr Ser Asn Val Ile Gly Thr Val Thr Ser Gly Lys Arg Lys Val -32 -30 -25
40	Tyr Leu Leu Ser Leu Leu Leu Ile Gly Phe Trp Asp Cys Val Thr Cys
	His Gly Ser Pro Val Asp Ile Cys Thr Ala Lys Pro Arg Asp Ile Pro
45	Met Asn Pro Met Cys Ile Tyr Arg Ser Pro Glu Lys Lys Ala Thr Glu . 20 25 30
50	Asp Glu Gly Ser Glu Gln Lys Ile Pro Glu Ala Thr Asn Arg Arg Val 45
50	Trp Glu Leu Ser Lys Ala Asn Ser Arg Phe Ala Thr Thr Phe Tyr Gln 55 60

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	H1 =		. Ala	: Asp	Se:	Ly:		n Asp	12.A. C	n Asy	> Asr 75	_	e Pro	ê Le	u Se:	F Pr
5	Let	se:	r ile	: Ser	Th:		a Phe	e Ala	Met	Th: 9(Lei	. CJ	y Ala	e Cyr	5 Ası 5
	Asp	Th:	r Leu) 61 n		Leu	u Met	: Glu	va) 105		: Lys	Phe	± Asj	7'n:		e 5e
10	61 u	Lys	Thr 115		Asp	G1r	o Ile	His 120		: Fhe	· Phe	Ala	Ly:) Asr	a Cys
	Arg	Leu 130	-	Yra	Lys	Аla	135		Ser	Ser	Lys	Leu 140		Ser	Ala	: Asr
75	Arg . 145		Phe	Gly	Asp	Ly·s 150		Leu	Thr	Phe	155		Thi	ተን ፣	Glr	160
	lle	Ser	Glu	Leu	Val 165	Туr	Giy	Ala	تړیه	Leu 170		Pro	Leu	Asp	Phe 175	Lys
20	Glu	Asn	Ala	Glu 180	Gln	Ser	Arg	Ala	Ala 185		Asn	L'ns	Trp	Vel 190		Asn
	Lys	Thr	Glu 195	-	Arg	Ile	Thr	Asp 200		Ile	Pio	Ser	Glu 205		Ile	: Asn
25	Glu	Leu 210		Val	Leu	Val	Leu 215	Val	Asn	Thr	lle	Туг 220	Phe	Lys	۲۱S	Leu
	Trp 225	Lys	Ser	Lys	Phe	5er 230		Glu	Asn	Thr	Arg 235	Lys	Glu	Leu	Phe	Tyr 240
30	Lys	Ala	Asp	Gly	Glu 245	Ser	Cys	5er	Ala	Ser 250	Met	Met	Tyr	Gln	G1 u 255	Gly
	Lys	Phe	Arg	Туг 260	Arg	Arg	Val	Ala	61 u 265	GŢ.'n	Thr	Gla	Val	Leu 270	Glu	Leu
35	Pro	Phe	Lys 275	Gly	Asp	Asp	Ile	Thr 280	Met	Val	Leu	Ile	Leu 285	Pro	Lys	Pro
	Glυ	Lys 290	Ser	Leu	Ala	Lys	Val 295	Glu	Lys	Gĵη	Leu	Thr 300	Pro	Glu	Val	Leu
40	Gln 305	G∑ n	Trp	Leu	Asp	Glu 310	Leu	Glu	G] u	Met	Met 315	Leu	Val	Val	His	Met 320
			-		325		•	•		330		-			335	Gln
45	Asp	Met	G] y	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	Glu	Lys ·	Ser	Lys 350	Leu	Pro
	G1 y	Ile	Val 355	Al a	Glu	G1 y	Жīд	Asp 360	Asp	Leu	Tyr	Va]	Ser 365	Asp	Ala	Phe
50		370					Val 375					380				
	Ser 385	Thr	Ala	Val		Leu 390	Trp	Pro	Arg		Leu . 395	4.s.n	Pro	.4. S Tı	Arg	Val 400

	5	Thr E	he L	ys A	la A 4	sn A 05	rg P	ro P	he L	ອນ V 4	al ?! 10	he I	le A	rg G		al Pro 15
5		eu A	sn T	h: I	le ĭ 20	le P	he M	et G	ly A 4	rg V 25	al Ai	la As	n P		ys Va 30	al Lys
10	(2) I	NFORI) SE((A) (B)	ON FO QUENC LENC TYPE TOPO	CE CH STH: E: an	LARAC 464 nino	CTER: amin	ISTIC no ac	cs:						
16		C.	ii) Þ	OLEC	CULE	TYPE	: pr	otei	n							
	W		xi) S													
20		-	~					- 2	5				-2	0		s Val
	T	/r Le -]	eu Le 15	u Se	r Le	u Le	u Le -1	u Il O	e Gl	y Ph	e Tr	P As		s Va	1 Th	r Cys
95	Hi	s Gl 1	y Se	r Pr	o Va	1 As 5	p Il	e Cy	s Th	r Al l	a Ly: O	s Pro	o Ar	g As	p Il	e Pro 5
25	Me	t As	n Pr	0 Me 2	t Cy	s Il	е Ту	r Ar	g Se 2	r Pr 5	o G1:	u Ly:	s Ly	s Ala 30		r Glu
	As ·	p Gl	u Gl 3	y Se 5	r Gl	n eJi	n Ly:	s Il	e Pr	o G1	u Ala	Th	: Ası) Ar	y Väl
30	Tr	p G1 5	u Lei O	u Se.	r Lys	s Ala	Asr 55	n Sea	r Ar	g Pho	e Ala	Thr 60	Th:	. Phe	Туг	Gln
35	Ні 6	s Le [.] 5	u Ala	a Asp	Ser	70	Asr	Asp	Asr	Asp	Asn 75	Ile	Phe	. Léu	Ser	Pro 80
00	Le	u Se.	r Ile	: Ser	Thr 85	Ala	Phe	: Ala	Met	Thr 90	Lys	Leu	G1 y	Ala	Cys 95	Asn
	Asi	Thi	r Leu	61n 100	Gln	Leu	Met	Glu	Val 105	Phe	Lys	Phe	Asp	Thr 110	Ile	Ser
40	Gl:	Lys	Thr 115	Ser	Asp	Gln	Ile	His 120	Phe	Phe	Phe	Ala	Lys 125	Leu	Àsn	Cys
	Arg	130	Tyr	Arg	Lys	Ala	Asn 135	Lys	Ser	Ser	Lys	Leu 140	Val	Ser	Ala	Asn
45	Arg 145	Leu	Phe	Gly	Asp	Lys 150	Ser	Leu	Thr	Phe	Asn 155	Glu	Thr	Tyr	Gln	Asp 160
	Ile	Ser	Glu	Leu	Val 165	Туr	Gly	Ala	Lys	Leu 170	Gln	Pro	Leu	Asp	Phe 175	Lys
50	Glu	Asn	Ala	Glu 180	Gln	Ser	Arg	Ala	Ala 185	Ile	Asn	Lys	Trp	Val 190	Ser	Asn

	Ly	s Th	: G] : 195		Arg	110	e Thi	Asp 200		116	: Fro	Se:			. 11	e Asn
	Glı		ı Tnı		Leu	val		. Vai		Tř: s	: Ile				G);	, Leu
5				: Lys	Phe				Asn	The				. Lev	ነ ምክፅ	≥ Tyr
	225 Lys		Asp	o Gly				Ser	љl a				Tyr	Gln		240 Gly
10	Lуs	Phe	Arg		245 Arg		Val	A.l.a		250 G1 y		Gln	Va]			Lev
	Pro	Phe			Asp	Asp	· Ile			Va]	Leu	Ile		270 Pro		Pro
75	Gl u				Ala	Lys		280 Glu		G1 u	Leu	Thr	285 Pro	Gl u	Val	Leu
	Gln	290 Glu		Leu	Asp	Glu	295 Leu	Glu	Glu	Met	Met	300 Leu		Val	His	Met
20	305 Pro		Phe	Ara	Ile	310 Glu		Glv	Phe	Ser	315 Leu	Lvs	G) u	Gln	Leu	320 Gln
				Leu	325					330		_			335	
25				340 Ala		_			345			-		350		
			355					360					365			
30		370		Phe			375				-	380				
	385			Val		390					395					400
35					405					410			_		415	
	Leu	Asn		11e 420	Ile	Phe	Met		Arg 425	Val	Ala .	Asn		Cys 430	Val	Lys
	(2)	INFO	PM4.T	ion :	FOR	SEQ	ID N	0: 1	7:							•
40		((A	EQUEI LEI TYI	NGTH PE: 8	: 46 amin	4 ат. о ас.	ino i id		s						
45				ECULI			-									
	Met			UENCE Asn \									Lýs A	Arg 1	Lys	Val
50	-32 Tyr	Leu 1	-30	Ser 1			-	-25				-	-20			
		-15										- 5				-

	H	is Gl	y Se	r Pro	Val	l Asp	o Ile	e Cy:	s Th	r Ala	a Lys	Pro	Ar	g Asi	- Il 1	e Pro
5	м	et As	n Pr	o Met 20	Cys	: Ile	Tyr	Arq	Se. 2	r Pro	Glu	Lys	Ly	5 Ala 30	Th.	r Glu
			•	-				41	•				4 5	•		g Val
10		J	•	ı Se <u>≓</u>			23					60				
	`			a Asp		70					75					80
15				e Ser	65					90					95)
				Gln 100					102					110		
20			113					120					125			
		150	•	Arg			135					140				
25				Gly		130					155					160
				Leu	105					170					175	
30				Glu 180					182					190		
			133	Gly .				200					205			
35				Val :			213					220				
				Lys 1	•	230					235					240
40					. 7 .					250					255	
				Tyr A 260				•	265				- 1	270		
45			2,3	Gly A			2	80				2	85			
				Leu A		2	95				3	00				
5 <i>0</i>				eu A		10		•		3	15				3	320
	rio	Arg :	rne A	rg I: 32	le G: 25	lu A	sp G	ly P	he S	er L 30	eu L	ys G	lu G		eu (35	ln

	Asp	ме:	G1	y Le: 34] As	r L-	u Ph	e Se 34		o	u Ly	s Se	r ly 35	s Le O	u Pr
s	CJ ?	· Ile	35!		≥ Gl	u Gl	y As	g As. 36		p Le	u Ty	r Va	1 Se 36	_	p Al	a Ph
	His	Lys 370		e Phe	e Le	ى Gl	υ Va 37		n 62	u Gl	u G)	y Se 38		u Al	a A.1	6 Al
10	Ser 385		: Ala	a Val	l Val	1 .la 390		e 61 :	y Ar	g Se.	r Le 39		n Pr	o As	n Ar	9 Va. 40
	Thr	Ph∈	: Lys	Ala	4.65 4.05		Pr:	o Phe	≥ Le	410		e Il	e Ar	g G1	u Va: 41:	
15	Leu	Asn	Thr	11e		≘ Ph∈	: Me	r 637	/ Arc	_	l Ala	a Ası	n Pro	6 Cy.	s Vai	l Lys
	(2)	INF	орма	MOIT.	FOF	SEQ	ID	NO:	15:							
20			t	A) L B) T	ENGT YPE:	CHA 'H: 4 ami OGY:	64 a	mino cid								
25						YPE: ESCR	•			ID N	0: 1	.8:				
	Met -32	Tyr	Ser -30	Asn	Vāl	Ile	Gly	Thr -25	٧ēl	Thr	Ser	G1 y	Lys -20		Lys	: Val
30	Tyr	Leu -15	Leu	Ser	Leu	Leu	Leu -10		61 y	Phe	Trp	Asp -5		Va 1	Thr	Cys
	His 1	Gly	Ser	Pro	Val 5	Asp	Ile	Cys	Thr	Ala 10	-	Pro	Arg	Asp	Ile 15	
35	Met	Asn	Pro	Met 20	Cys	Ile	Tyr	Arg	Ser 25	Pro	Glu	Lys	Lys	Ala 30	Thr	Glu
	Asp	Glu	Gly 35	Ser	Glu	Gln	Lys	11e 40	Pro	Glu	Ala	Thr	Asn 45	Arg	Arg	Val
40	Trp	61 u 50	Leu	Ser	Lys	Άla	Asn 55	Ser	Arg	Phe	Ala	Thr	Thr	Phe	Tyr	Gln
	His 65	Leu	Ala	Asp	Ser	Lys 70	Asn	Asp	Asn	Asp	Asn 75	Ile	Phe	Leu	Ser	Pro 80
15	Leu	Ser	lle	Ser	Th: 85	Ala	Phe	.la	Met	Thr 90	Lys	Ŀeυ	Сlу	Άla	Cy's 95	Asn
SO	Asp	Thr	Leu	Gln 100	Gln	Leu	Met	Glu	Val 105	Phe	Lys	Phe	Asp	Thr 110	11e	Ser
	Glu		Thr 115	Ser.	Asp	Gln	11€	His 120	Phe	Phe	P'ne	A.la	Lys 125	Leu	A.s.n	Cys

	Ar	g Le 13	u Ту О	r Ar	g Ly	s Al	а Лs 13	n Ly	's Se	r Se	= Ly	s Le		1 Se	r Al	a Asn
5	Ar 14	g Le 5	u Ph	e Gl	y As	p Ly 15	s Se O	r Le	u Th	r Ph	e As 15	n G1 5	u Th	r Ty	r Gl	n Asp 160
	11	e Se	r Gl	u Le	16	1 Ту 5	r Gl	y Al	a Ly	s Le	u Gl 0	n Pr	o Le	u As	p Ph 17	e Lys 5
10	G1	u As:	n Al	a Gl:	G1;	n Se	r Ar	g Al	a &1 18	a Ile 5	e As	n Ly	s Tr	p Va 19	1 Se 0	r Asn
	Ly:	s Th	19:	u Gly 5	/ Arc	g Ile	e Th	20	p Va. O	1 116	Pr	o Se	z Gl: 20:		a Il	e Asn
15	Gl	210	ı Th:	r Val	. Lei	Val	l Let 21	u Vai 5	l As	n Th:	: Il	e Ty.	r Phe	e Ly	s Gl	y Leu
	Try 225	Lys 5	S Se	t Lys	Phe	230	Pro	o Glu	u A.sı	n Thr	23:	Ly:	s Glu	u Le	u Phe	Tyr 240
20	Lys	Ala	Asp	o Gly	Glu 245	Ser	Cys	S Se :	r Ala	Ser 250	Met	: Met	ту	Gl:	n Glu 255	Gly
	Lys	Phe	Arg	7 Tyr 260	Arg	Arg	y Val	Ala	Glu 265	ı Gly	Thi	: Glr	val	Le:		Leu
25	Pro	Phe	Lys 275	Gly	Asp	Asp	Ile	280	Met	: Val	Lev	116	285		Lys	Pro
25	Glu	Lys 290	Ser	Leu	Ala	Lys	Val 295	Glu	Lys	G)u	Leu	7'n,1	Pro	Glu	ı Val	Leu
	Gln 305	Glu	Trp	Leu	Asp	Glu 310	Leu	Glu	Glu	Met	Met 315	Leu	Val	. Val	His	Met 320
30	Pro	Arg	Phe	Arg	Ile 325	Glu	Asp	G1 y	Phe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	
	Asp	Met	Gly	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	Glu	Lys	Ser	Lys 350		Pro
35	Gly	Ile	Val 355	Ala	Glu	Gly	Arg	Asp 360	Asp	Leu	Tyr	Val	Ser 365	Asp	Ala	Phe
	His	Lys 370	Ala	Phe	Leu	Glu	Val 375	Asn	Glu	Glu	G1 y	5er 380	Glu	Ala	Ala	Ala
40	Ser 385	Thr	Ala	Val	Val	Ala 390	Leu	Gly	Arg	Ser	Leu 395	Asn	Pro	Asn	Arg	Val 400
	 Thr	Phe	Lys	Ala :	Asn 405	Arg	Pro	Phe	Leu	Val 410	?he	lle	Arg	Glu	Val 415	Pro
45	Leu	Asn	Thr	11e 420	Ile	Pt.e	Met	Gly	Arg 425	Val .	Ala	Asn	Pro	Cys 430	Val	Lys
	(2)	INFO	RMAT.	ION :	FOR :	SEQ :	ID N	0: 1	9:							

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 464 amino acids

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					TPE: OPO1											
5					LE T		•			ו פו	NO:]	15:				
	Me t - 32	Туг		. Asn					: Val				y Lys -20		, Lys	Va.
70	туз	Leu -15		Ses	Leu	Leu	-10		. G1	Phe	. Tsp	Asp -3		: Val	The	Cy:
	His 1	-	Ser	Pro	Va <u>1</u> 5	Asp	lle	Сув	Thr	Ala 10		Pro	Arç) Asp) I)e	
75	Met	Asn	Pro	Met 20		Ile	Туг	Arg	Ser 25		G) u	Lys	Lys	Ala 30	Thr	G2:
	Asp	Glu	61 y 35		Glu	G) n	Lys	11e 40		Glu	Ala	T'nı	Asn 45		Arg	Ve)
20	Trp	Gl ນ 50		Ser	Lys	Ala	Asn 55		Arg	Phe	Ala	Thr 60		Phe	Туг	Gln
	His 65		Ala	Asp	Ser	Lys 70		Asp	Asn	Asp	Asn 75		Phe	Leu	Ser	Pro
25	Leu	Ser	Ile	Ser	Thr 85	Ala	Phe	Ala	Меt	Thr 90		Leu	G] y	Ala	Cys 95	
	Asp	Thr	Leu	Gln 100	Gln	Leu	Met	Glu	Val 105	Phe	Lys	Phe	Asp	Thr 110	Ile	Ser
30 ·	Glu	Lys	Thr 115	Ser	Asp	Gln	Ile	His 120	Phe	Phe	Phe	Ala	Ly's 125		Asn	Cys
	Arg	Leu 130	Tyr	Arg	Lys	₽la	Asn 135	Lys	Ser	Ser	Lys	Leu 140	Vāl	Ser	řlа ·	Asn
35	145				-	150					155				G] n	160
					165					170					Phe 175	
40				180					185					190	Ser	
	-		195	•	_			200	•				205		Ile	
4 5		210					215					220			Gly	
	225					230					235	_			Phe	240
50	_				245		-			250			_		Glu 255	_
	r),2	r 116	~1.g	260	arg	∴± g	vsī	W 1 E	265	er A	Int	GID	val	270	G) u	⊾eu

	Pro Phe Lys Gly Asp Asp Ile Thr Met Val Leu Ile Leu Pro Lys Pro 275 280 285
5	Glu Lys Ser Leu Ala Lys Val Glu Lys Glu Leu Thr Pro Giu Val Leu 290 295 300
	Gln Glu Trp Leu Asp Glu Leu Glu Glu Met Met Leu Val Val His Met 305 310 315 320
10	Pro Arg Phe Arg Ile Glu Asp Gly Phe Ser Leu Lys Glu Gln Leu Gln 325 330 335
	Asp Met Gly Leu Val Asp Leu Phe Ser Pro Glu Lys Ser Lys Leu Pro 340 345 350
15	Gly Ile Val Ala Glu Gly Arg Asp Asp Leu Tyr Val Ser Asp Ala Phe 355 360 365
	His Lys Ala Phe Leu Glu Val Asn Glu Glu Gly Ser Glu Ala Ala Ala 370 375 380
20	Ser Thr Ala Val Val Gly Leu Gly Arg Ser Leu Asn Pro Asn Arg Val 385 390 395 400
	Thr Phe Lys Ala Asn Arg Pro Phe Leu Val Phe Ile Arg Glu Val Pro 405 410 415
25	Leu Asn Thr Ile Ile Phe Met Gly Arg Val Aia Asn Pro Cys Val Lys 420 425 430
	(2) INFORMATION FOR SEQ ID NO: 20:
30	(i) SEQUENCE CHARACTERISTICS:(A) LENGTH: 464 amino acids(B) TYPE: amino acid(D) TOPOLOGY: linear
35	(ii) MOLECULE TYPE: protein
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20: Met Tyr Ser Asn Val Ile Gly Thr Val Thr Ser Gly Lys Arg Lys Val -32 -30 -25
40	Tyr Leu Leu Ser Leu Leu Leu Ile Gly Phe Trp Asp Cys Val Thr Cys -15 -25 Tyr Leu Leu Ser Leu Leu Leu Ile Gly Phe Trp Asp Cys Val Thr Cys -5
	His Gly Ser Pro Val Asp Ile Cys Thr Ala Lys Pro Arg Asp Ile Pro 1 5 10 15
45	Met Asn Pro Met Cys Ile Tyr Arg Ser Pro Glu Lys Lys Ala Thr Glu 20 25 30
	Asp Glu Gly Ser Glu Gln Lys Ile Pro Glu Ala Thr Asn Arg Arg Val 35 40 45
50	Trp Glu Leu Ser Lys Ala Asn Ser Arg Phe Ala Thr Thr Phe Tyr Gln 50 55 60

	His 65		⊁.la	Asp	Ser	Lys 70		. Asp	, Asr	. Asp	Asn 75		: Pire	Leu	Ser	Pro 90
5	Leu	Ser	1)e	Ser	Thr E5		Phe	Ala	Met	Thr 90		Leu	61 y	Ala	Cys 95	Asn
	Asp	The	Leu	61n 100		Lev	Met	Glu	Va) 105		Lys	Phe	Asp	The 110		Ser
10	G] u	Lys	Thr 115	Ser	Asp	Gl n	lle	His 120		Phe	Phe	Ala	Lys 125		Asn	Cys
	Arg	Leu 130		Arg	Lys	Ala	Asn 135		Ser	Ser	Lys	L∈u 140		Ser	Ыz	Asn
15	145	Leu	Phe	Gly	Asp	Lys 150	Ser	Leu	Thr	Phe	Asn 155	Glu	Thr	туг	Gln	A.sp 160
	Ile	Ser	Glu	Leu	Val 165	Tyr	6 1 አ	Ala	L)'s	Leu 170	Gln	Pro	Ŀeu	Asp	Phe 175	Lys
20	Glu	Asn	Ala	Gl u 180	Gln	Ser	Arg	<u>Al</u> a		Ile	Asn	Lys	Trp	Val 190	Ser	Asn
	Lys	Thr	Glս 195	Gly	УLÔ	Ile	Thr	Asp 200		Ile	Pro	Ser	Glս 205	Ala	Ile	Asn
25	Glu	Leu 210	Thr	Val	Leu	Val	Leu 215	Val	Asn	Thr	Ιlε	Tyr 220	Phe	Lys	e3 A	Leu
	Trp 225	Lys	Ser	Lys	Phe	Ser 230	Pro	Glu	Asn	Thr	Arg 235	Lys	Glu	Leu	Phe	Tyr 240
30	Lys	Ala	Asp	Gly	Glu 245	Ser	Cys	Ser	Ala	Ser 250	Met	Met	Tyr	Gju	Gl u 255	Gly
	L)'S	Phe	Arg	Tyr 260	Arg	Arg	Val	Ala	Gl u 265	Glу	Thr	Gl'n	Val	Leu 270	Glu	Leu
35			275					280		Val			285			
		290					255			Glu		300				
40	305					310				Met	315					320
		-			325		-			Ser 330					335	
45				340				٠	345	Pro				350		
			355					360		Leu			365			
50		370					375			Glu		380				
	Ser 385	Thr	Ala	Val		11e 390	Ala	61 Y	Æгg	Ser	Leu 395	Asn	Pro	Asn.	Arş	Val 400

	Thr Phe Lys Ala Asn Arg Pro Phe Leu Val Phe Ile Arg Glu Val Pro 405 410 425
5	Leu Asn Thr Ile Ile Phe Met Gly Arg Val Ala Asn Pro Cys Val Lys 420 425 430
	(2) INFORMATION FOR SEQ ID NO: 21:
10	 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 464 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear
	(ii) MOLECULE TYPE: protein
15	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:
	Met Tyr Ser Asn Val Ile Gly Thr Val Thr Ser Gly Lys Arg Lys Val -32 -30 -25 -20
20	Tyr Leu Leu Ser Leu Leu Leu Ile Gly Phe Trp Asp Cys Val Thr Cys
	His Gly Ser Pro Val Asp Ile Cys Thr Ala Lys Pro Arg Asp Ile Pro 1 5 10 15
25	Met Asn Pro Met Cys Ile Tyr Arg Ser Pro Glu Lys Lys Ala Thr Glu 20 25 30
	Asp Glu Gly Ser Glu Gln Lys Ile Pro Glu Ala Thr Asn Arg Arg Val 35 40 45
30	Trp Glu Leu Ser Lys Ala Asn Ser Arg Phe Ala Thr Thr Phe Tyr Gln 50 55 60
	His Leu Ala Asp Ser Lys Asn Asp Asn Asp Asn Ile Phe Leu Ser Pro 65 70 75 80
35	Leu Ser Ile Ser Thr Ala Phe Ala Met Thr Lys Leu Gly Ala Cys Asn 85 90 95
	Asp Thr Leu Gln Gln Leu Met Glu Val Phe Lys Phe Asp Thr Ile Ser 100 105 110
40	Glu Lys Thr Ser Asp Gln Ile His Phe Phe Phe Ala Lys Leu Asn Cys 115 120 125
	Arg Leu Tyr Arg Lys Ala Asn Lys Ser Ser Lys Leu Val Ser Ala Asn 130 135 140
4 5	Arg Leu Phe Gly Asp Lys Ser Leu Thr Phe Asn Glu Thr Tyr Gln Asp 145 150 155 160
43	Ile Ser Glu Leu Val Tyr Gly Ala Lys Leu Gln Pro Leu Asp Phe Lys 165 170 175
50	Glu Asn Ala Glu Gln Ser Arg Ala Ala Ile Asn Lys Trp Val Ser Asn 180 185 190
5 <i>0</i>	Lys Thr Glu Gly Arg Ile Thr Asp Val Ile Pro Ser Glu Ala Ile Asn 195 200 205

		Leu 210	The	Vál	Leu	Val	Leu 215		Asn	Thr	:3e	220 27.1		۲۷s	Gly	Le 2
5	Trp 225	Lys	Ser	Ly's	Phe	S∈r 230		G] v	Asn	Thr	Arg 235	ly:s	G) u	Leu	Phe	Tyr 240
	Lys .	<i>I</i> .] a	Asp	61 y	Glu 245	Ser	Cys	Ser	A.la	Ser 250	Met	Met	Туг	Gjn	Glu 255	61 y
10	Lys	Phe	Ъrg	Tyr 260	Arg	Arg	Val	Ala	Glu 265	6) À	Thr	Gln	Val	Leu 270	Glu	Leu
	Pro :		Lys 275	Gly	Asp	Asp	Ile	Thr 280	Met	Val	Leu	Ile	Leu 285	Pro	Lуs	Pro
15		Lys 290	Ser	Leu	Ala	Lys	Val 295	G1 u	Lys	Glυ	Leu	Thr 300	Pro	Glu	Val	Leu
	Gln (305	Glu	Trp	Leu	Asp	Gl u 310	Leu	Glu	Glu	M÷t	Met 315	Leu	Val	Val	His	Het 320
20	Pro J	Arg	Phe	Arg	11e 325	Glυ	Asp	ΘĴУ	Phe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	Gln
	Asp h	Met	Gly	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	Glu	Lys	Ser	Lys 350	Leu	Pro
25	Gly 1		Val 355	Ala	G1 u	ej À	Arg	Asp 360	Asp	Leu	Tyr	Vāl	Ser 365	Asp	Ala	Phe
	His I	Lys . 370	Ala	Phe	Leu	Glu	Val 375	Asn.	Glu	Gl u	G1 y	Ser 380	Glu	<i>F</i> .la	Ala	Ala
30	Ser T 385	Thr .	Ala [.]	Val		11e 390	Ala	Gly	Arg		Leu 395	Asn	Pro	Asn	Arg	Val 400
	Thr F	Phe :	Lys		405		Pro	Phe		Val 410	Phe	Ile	Arg	Glu	Val 415	Pro
35	Leu A	Asn '	Thr	Il∈ 420	lle	Phe	Met		Arg 425	٧al	Ala	Asn	Pro	Cys 430	Val	Lys
	(2) I	(NFO	RMAT	NOI	FOR	SEQ	א סו	0: 2	2:							
40		(:	(A.) LE	NCE NGTH PE: POLO	: 46 amin	4 am	ino . id		s						
45					E TY											
·	Met T		_		E DE. Val :								Lys .	Łrg'.	ris ,	Val
50	-32 Tvr L	-	- 30				•	-25				_ ,	- 20	_		
•	-	15					- 10		-			- 5	-			-

	His Gly Ser Pro Val Asp Ile Cys Thr Ala Lys Pro Arg Asp Ile Pro 1 5 10 15
5	Met Asn Pro Met Cys lle Tyr Arg Ser Pro Glu Lys Lys Ala Thr Glu 20 25 30
	Asp Glu Gly Ser Glu Gln Lys Ile Pro Glu Ala Thr Asn Arg Arg Vel 35 40 45
10	Trp Glu Leu Ser Lys Ala Asn Ser Arg Fhe Ala Thr Thr Phe Tyr Gln 50 55 60
	His Leu Ala Asp Ser Lys Asn Asp Asn Asp Asn Ile Phe Leu Ser Pro 65 70 75 80
15	Leu Ser Iie Ser Thr Ala Phe Ala Met Thr Lys Leu Gly Ala Cys Asn 85 90 95
	Asp Thr Leu Gln Gln Leu Met Glu Val Phe Lys Phe Asp Thr Ile Ser 100 105 110
20	Glu Lys Thr Ser Asp Gln Ile His Phe Phe Phe Ala Lys Leu Asn Cys 115 120 125
	Arg Leu Tyr Arg Lys Ala Asn Lys Ser Ser Lys Leu Val Ser Ala Asn 130 135 140
25	Arg Leu Phe Gly Asp Lys Ser Leu Thr Phe Asn Glu Thr Tyr Gln Asp 145 150 155 160
	Ile Ser Glu Leu Val Tyr Gly Ala Lys Leu Gln Pro Leu Asp Phe Lys 165 170 175
30	Glu Asn Ala Glu Gln Ser Arg Ala Ala Ile Asn Lys Trp Val Ser Asn 180 185 190
	Lys Thr Glu Gly Arg Ile Thr Asp Val Ile Pro Ser Glu Aia Ile Asn 195 200 205
35	Glu Leu Thr Val Leu Val Leu Val Asn Thr Ile Tyr Phe Lys Gly Leu 210 215 220
	Trp Lys Ser Lys Phe Ser Pro Glu Asn Thr Arg Lys Glu Leu Phe Tyr 225 230 235 240
40	Lys Ala Asp Gly Glu Ser Cys Ser Ala Ser Met Met Tyr Gln Glu Gly 245 250 255
	Lys Phe Arg Tyr Arg Arg Val Ale Glu Gly Thr Gln Val Leu Glu Leu 260 265 270
45	Pro Phe Lys Gly Asp Asp Ile Thr Met Val Leu Ile Leu Pro Lys Pro 275 280 285
	Glu Lys Ser Leu Ala Lys Val Glu Lys Glu Leu Thr Pro Glu Val Leu 290 295 300
50	Gln Glu Trp Leu Asp Glu Leu Glu Glu Met Met Leu Val Val His Met 305 310 315 320
	Pro Arg Phe Arg Ile Glu Asp Gly Phe Ser Leu Lys Glu Gln Leu Gln 325 330 335

	A:	sp M	et G	ly i 3	€U V. 40	el A	sp P	eu P		Ser F 245	ro G	l of	ביני.	er L 3	}'s L	eu P	ŗ
s	G.	ly 11	le V. 35	1 A.	la G	lu G.	ly A	rg A 3	sp / 60	sp l	.ėυ T	yr V		er A 65	sp A	la P	'n
	ні	is Ly 37	rs Al 70	la Pi	ne Le	ev G)บ Va 31	al A 75	sn G	10 G	lu G		er G 80	lu A	l e ይ	la A	1
10	S 6	r Th	r Al	a Pì	ne Va	39 11 [1	le A] 0	la G	A 12.	rg S		eu A 95	EN P	ro A	sn A	rg V 4	
	Th	r Ph	e Ly	s Al	a As 40	n Ar 15	9 P1	(o P)	he L		al Pi 10	he I.	le A	rg Gl	lu Va 41		r
15	Le	u As	n Th	r I1 42	e Il 0	e Ph	e Me	t Gl		rg V: 25	al Al	la As	sn Pi	co C; 43		sl L	5 7 :
	(2) IN	FORM	ATIO	N FO	R SE	O ID	NO:	23:	:							
20				(A) (B)	UENC LENG TYPE TOPO:	TH: : am	464 . ino :	amin acid	o ac								
25					NCE 1		•			חדה	NO.	22.					
	Met -32	Ty:		. Ası	val				r Va				у Ly -2		g Ly	s Va	1
30	7 yr	Leu -15	Leu S	se:	Lev	l Leu	. Leu -10	: 11e	e Gl	y Ph	e Tr	o Ası	р Су: 5	s Val	1 Th	e Cy	s
	His 1	G13	Ser	Pro	Val 5	Asp	lle	: Cys	s Th.	r Ala 10		s Pro	o Asq	g Asp) Ile 1:		0
35	Met	. Asn	Pro	Ме ⁻ т 20	Cys	Ile	Туг	Arg	3 Se: 2:		o Glu	Ly:	5 Lys	* Ala 30		c Gl	υ
			35		Glu			40	}				4 5	•			
40	Trp	Gl v 50	Leu	Ser	Lys	Ala	Asn 55	Ser	Asç	Phe	: Ala	Thr 60		Phe	Tyr	Gl i	3
	65				Ser	70					75					80)
4 5					Thr 85			-		50					95		
				100	Gln				105					110			
50			112		Asp			120					125			_	
~	Arg	Leu 130	Tyr	Arg	Ly s	Ala	Asn 135	Lys	Ser	Ser	Lys	Leu 140	Val	Ser	Ala	Asn	

	Ar 14	g Let 5	u Pho	e Gly	y As _i	P Lys	s Se	r Le	u Th	r Ph	Ası 155	n G1	u Th	г Ту	r Gl	n Asp 160
5	110	e Sei	r Glu	ı Let	Va: 165	1 Tyı 5	G1;	y Ala	a Ly	s Let 170) i G]t	Pr	o Le	u Asj	Phe 17	e Lys 5
	Glv	: Asr	n Ala	180	Glr	n Ser	Arq	Ala	a Al 18	a Ile 5	. Asr	Ly	s Tr	9 Va. 190	l Se	: Asn
10	Lys	Thi	Glu 195	Gly	' Arg	Ile	The	200	o Va.	l lle	Pro	Sei	r Glu 205		a Ile	Asn
	Glu	210	Thr	Val	Lev	ı Val	Leu 215	Val	Ası	n Thr	Ile	Ty:		Ly:	Gly	/ Leu
15	Trp 225	Lys	Ser	Lys	Phe	Ser 230	Pro	61 u	Ası	n Thr	Arg 235	Lys	Glu	Leu	Phe	Tyr 240
	Lys	Ala	Asp	Gly	Glu 245	Ser	Cys	Ser	Ala	Ser 250	Met	Met	туг	Glm	Glu 255	
20	Lys	Phe	Arg	Туг 260	Arg	Arg	Val	Ala	Glu 265	Gly	Thr	Gln	Val	Leu 270		Leu
20	Pro	Phe	Lys 275	eJà	Asp	Asp	Ile	Thr 280	Met	: Val	Leu	Ile	Leu 285		Lys	Pro
		290					295			Glu		300	1			
25	303					310				Met	315					320
					325					Ser 330					335	
30				340					345	Pro				350		
			355					360		Leu			365			
35		370					375			Glu		380				
	303					350					395					400
10	•				405					Val 410					415	
	Leu /	Asn '	Thr	11e : 420	lle	Phe 1	Met	Gly .	Arg 425	Val .	Ala .	A.s n	Pro	Cys 430	Vaļ	Lys
5	(2) 3	INFO	RMAT:	ION I	FOR S	SEQ 1	D NO): 2	4:							
			L) SE	EQUEN	ICE (CHARA : 464	CTE	RIST:	ICS:	s						
o						mino Y: 1										

(ii) MOLECULE TYPE: protein

			(xi	.) SE	QUEN	ICE D	ESCI	RIPT)	ON:	SEQ	ID)	10: 3	4:				
s		Met - 32		- Ser		Val	Ile	e Gly	7hr		Thi	r Sei	. 6 17	· Lys -20	. Asg	Lys	; Val
		Tyr	Leu -15		Ser	Leu	Leu	-10		G1 y	Phe	Trp	Asp -5	Cys	Va]	Thr	יעט:
10		His 1	ej A	Ser	Pro	Va 1 5	Asp	lle	Cys	Thr	Ala 10		Pro	Arg	Asp	11e	
		Met	Asn	Pro	Met 20	Cys	lle	Tyr	Arg	Ser 25	Pro	Glu	Lys	Lys	Ala 30		GJ n
75		Asp	Glu	Gly 35	Ser	Glu	Gln	Lys	11e 40	Pro	Glu	Ala	Thr	Asn 45	Arg	Arg	Val
		Trp	Glu 50	Leu	Ser	Lys	Ala	Asn 55	Ser	Arg	Phe	Ala	Thr 60	Thr	Phe	Туr	Gln
20	•	His 65	Leu	Ala	Asp	Ser	Lys 70	Asn	Asp	Asn	Asp	Asn 75	11e	Phe	Leu	Ser	Pro 80
		Leu	Ser	Ile	Ser	Thr 85	Ala	Phe	Ala	Met	Thr 90	Lys	Leu	G1 y	Ala	Cys 95	Asn
25		Asp	Thr	Leu	Gln 100	Gln	Leu	Met	Glu	Val 105	Phe	Lys	Phe	Asp	Thr 110	Ile	Ser
	•	Glu	Lys	Thr 115	Ser	Asp	Gln	Ile	His 120	Phe	Phe	Phe	Ala	Lys 125	Leu	Asn	Cys
30			Leu 130	Tyr	Arg	Lys	Ala	Asn 135	Lys	Ser	Ser	Lys	Leu 140	Val	Ser	Ala	asn
		Arg 145	Leu	Phe	GJ À		Lys 150	Ser	Leu	Thr	Phe	Asn 155	Glu	Thr	Туr	Gln	Asp 160
35		Ile	Ser	Glu	Leu	Val	Tyr	Gly	Ala	Lys	Leu	Gln	Pro	Leu	Asp	Phe	Lys

180 185 190

Lys Thr Glu Gly Arg Ile Thr Asp Val Ile Pro Ser Glu Ala Ile Asn 200

Glu Leu Thr Val Leu Val Leu Val Asn Thr Ile Tyr Phe Lys Gly Leu 215

Trp Lys Ser Lys Phe Ser Pro Glu Asn Thr Arg Lys Gly Leu 230

Lys Ala Asp Gly Glu Ser Cys Ser Ala Ser Met Met Tyr Gln Glu Gly

Lys Phe Arg Tyr Arg Arg Val Ala Glu Gly Thr Gln Val Leu Glu Leu 260 265 270

250

Glu Asn Ala Glu Gln Ser Arg Ala Ala Ile Asn Lys Trp Val Ser Asn

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	F	Pro Ph	ne Lys 275	s Gly 5	Asp	Asp	Ilė	Thr 290	Met	Val	Leu	Ile	Leu 285	Pro	Lys	Pr
5	G	lu Ly 29	s Sei 10	r Leu	Ala	Lys	Val 295	Glu	Lys	Gl v	Leu	Thr 300	Pro	Glu	Val	Lei
	G 3	ln Gl 05	u Trp	Leu	Asp	Glu 310	Leu	Glu	Glu	Met	Met 315	Leu	Va 1	Val	His	Met 32(
10		ro Ar			323					330					335	
	А	sp Me	t Gly	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	Glu	Lys	Ser	Lys 350	Leu	Pro
15		ly Il	333					360					365			
		is Ly: 37	•				3/5					380				
20						390					395		<i>.</i>			400
		r Phe			405					410					415	
25	Le	u Asr	Thr	11e 420	Ile	Phe	Met	Gly A	Arg 425	Val .	Ala .	Asn		Cys 430	Val	Lys
	(2) INF	ОРМАТ	rion	FOR	SEQ	ID N	0: 2:	5:							
30			(E	EQUE () LE () TY () TO	NGTH PE:	: 464 amino	4 am.	ino a id	ICS:	5						
	, .) MOL													
35	Me 1 - 32	Tyr	Ser . -30				ly 1	hr V					ys A	rg I	Lys \	/al
	•	- : Leu -15	50			eu L	-	25			rp A	-	20			
40	His	Gly	Ser 1	Pro V	'al A 5			ys Ti	hr A	la L 10		_	rg A		le F 15	ro
	·.· Met	Asn	Pro N	1et C 20	ys I	le T	yr Ά	rg Se			lu L	ys L				lu
45	Asp	Glu	Gly s 35	Ger G	lu G	ln L	ys I	le Pr 40	6 G	lu Al	la Ti				rg V	al
	Trp	Glu : 50	Leu S	er L	ys A	la As	sn Se 55	er Ar	g Ph	e Al	a Tr	r T)	nr Pl	ne T	yr G	ln
50	His 65	Leu)	Ala A	sp Se	er Ly	/s As 70	in As	p As	n As	p As	n Il 5	e Pl	ne Le	eu Se		r o 3 0

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	Lei	u Se	r Il	e Sei	r Thi		a Ph	e A1	a Me		r Ly	s Lė	u Gl	y Al	а Су. 9	s Asn 5
5	Asj	o Th.	r Lei	ن Glr 100		ı Lei	u Me	t Gl	u Va 10		e Ly	s Ph	e As	р Th 11		e Ser
	Glı	· Ly·	115		: Asp	o Gla	o Il	e Hi 12		e Ph	e Pir	e Al	a Ly. 12		u Ası	r Cys
10	Arg	le: 130		: Arg	ιβλε	Ala	135		s Se	r S÷	r Ly:	s Le		l Se	r Ala	a Asn
	Arg 145		Phe	: Gly	Asp	Lys 150		r Le	u Th	r Ph	e Ası 159		מלד ט	ту:	r Glr	160
15	Ile	: Ser	Glu	Leu	Val 165		G1,7	/ Ala	a Ly	s Le		Pro) Lev	As,	2 Phe 175	Lys
	Glu	Asn	Ala	Glu 180		Ser	Arg	, Ala	18:		≙ Asr	Lys	Tr	Va]		Asn
20	Lys	Thr	Glu 195		Arg	lle	Thr	200		1116	Pro	Ser	61u 205		Ile	Asn
	G1 u	Leu 210		Val	Leu	Val	Leu 215		. Ası	The	: Ile	Tyr 220		Lys	Gly	Leu
25	Trp 225	Lys	Ser	Lys	Phe	Ser 230	Pro	Glu	Asr	Thr	235		Glu	Leu	Phe	Tyr 240
					245					250	1				Glu 255	
30				260					265	,				270		
			275					280					285		Lys	
35		290					295	•				300			Val	
	305					310					315				His	320
40					325					330		•			Leu 335	
				340					345					350	Leu	
45			355		•			360					365		Ala	
		370					375					380			Ala	
50	385				:	390					395				Arg	400
		. ,,,,	<i>-</i>	nie j	405	19 i	. TO	: HE	TEU	410	rne	11 E	AI G		Val 415	rro

Leu Asn Thr Ile Ile Phe Met Gly Arg Val Ala Asn Pro Cys Val Lys 420 425 430

3	(:	5) IV	FORM	MTIC	N FO	R SE	QID	HO:	26:							
10			(i)	(A) (B)	LENG TYPE	E CH TH: : am LOGY	464 ino	amin acid	o ac	S: ids						
		(i	i) M	OLEC	ULE	TYPE	: pr	otei	n							
		(%	i) s	EQUE	NCE	DESC	RIPT	ION:	SEQ	ID :	NO:	26:				
15	Me - 3	t Ty 2	r Se -3	r As 0	n Va	1 11	e Gl	у Th -2	r Va 5	l Th	r Se	r Gl	y Ly -2		J Lys	s Vai
	Ту	r Le	u Lei 5	u Se	r Le	u Lei	Le	u Il O	e Gl	y Phe	e Trj	P As	р Су: 5	s Val	Thi	cy:
20	Hi	s Gl	y Se	r Pro	o Va	l Asp 5	o Il	е Су:	s Th	r Ala	a Ly:	s Pro	o Ar	g Asp) Ile	
				21	,	s Ile			23	•				30)	
25	As	p Glu	3 Gl y	, 2e1	c Glu	ı Glr	Lys	5 Ile 40	e Pro	Glu	a Ala	1 Th	r Ası 45		Arç	/Val
	Trj	9 Glu 50	ı Leu)) Sei	Lys	: Ala	Asr 55	n Ser	Arq	Phe	: Ala	Th.:		Phe	Туг	Glr
30	His 65 	s Lev	Ala	Asp	Ser	Lys 70	Asn	Asp) Asr	Asp	Asn 75		Phe	. Leu	Ser	Pro 80
	Let) Ser	: Ile	Ser	Thr 85	Ala	Phe	Ala	Met	Thr 90	Lys	Leu	G1;	Ala	Cys 95	Asn
35	Asp	Thr	Leu	Gln 100	Gln	Leu	Met	Glu	Val 105	Phe	Lys	Phe	Asp	Thr 110	Ile	Ser
	Glu	Lys	Thr 115	Ser	Asp	Gln	Ile	His 120	Phe	Phe	Phe	Ala	Gln 125	Leu	Asn	Cys
40	Arg	Leu 130	Tyr	Arg	Lys	Ala	Asn 135	Lys	Ser	Ser	Lys	Leu 140	Val	Ser	Ala	Asn
	Ťán					Lys 150				Ť	155	•				160
45	Ile	Ser	Glu	Leu	Val 165	Tyr	Gly	Ala	Lys	Leu 170	Gln	Pro	Leu	Asp	Phe 175	Lys
				100		Ser			185					190		
50	Lys	Thr	Glu 195	G1 y	Arg	Ile	Thr	Asp 200	Val	Ile	Pro	Ser	Glu 205	Ala	Ile	Asn

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Alberta Comp. Let a decreasion and a let

	G	lo La	eu Th !C	r Val	Lét	va]	l Let 215	ıVa)	Asn	Thr	3 l e	Ty1		· Lys	ely	Leu
5	T: 22	ep L <u>y</u> 25	s Se	r Lys	Phe	Ser 230	Pro	• Glu	A.5 N	Th:r	Arg 235		G)ı	. Leu	. Phe	Tyr 240
	L	'E 7]	a As _i	p Gly	G1u 245	Ser	Cys	Ser	Ala	Ser 250	Met	Met	Tyr	Gln	G) u 255	
10	ГŻ	's Ph	e Arç	Tyr 260	Arg	Arg	Val	Ala	61 u 265	GJ Y	Thr	Gln	Va]	Ն∈ս 270		Leu
	Pr	o Ph	e Lys 275	613.	Asp	Asp	Ile	Thr 280	Met	Val	Leu	Ile	Նես 285	Pro	Lys	Pro
15	Gl	n Th		: Leu	Ala	Lys	Val 295	Glu	Lλίε	Gl v	Leu	Thr 300	Pro	Gl u	Val	Leu
	G1 30	ກ Gl: 5	u Trp	Leu	Asp	Gl u 31 0	Leu	Сĵл	61 n	Met	Met 315	Leu	Val	Val	His	Met 320
20	Pr	o Ar	g Phe	Arg	Ile 325	Glu	Æsр	Gly	Phe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	Gln
	Ası	p Met	: Gly	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	6) n	Ly's	Ser	Lys 350	Ŀeυ	Pro
25	G1	y Ile	255 355	Ala	Glu	G1 y	Arg	Asp 360	Àsp	Leu	Tyr	Val	5er 365	Asp	Ala	Phe
	His	370	Ala	Phe	Leu	Glu	Val 375	Asn	Gl v	Glu		S€1 380	Glu	Ala	Ala	Ala
	5e1 385	Thr	Ala	Val	Vāl	Ile 390	Ala	Pro .	Arg		Leu . 395	Asn	Pro	Asn		Val 400
30	Thr	Phe	Lys	Ala	Asn . 405	Arg	Pro	Phe :		Val : 410	Phe :	īle :	Arg		Val 415	Pro
	Leu	Asn	Thr	Ile 420	Ile '	Phe :	Met (trg \ 125	√al 3	Ala)	Asn.		Cys 430	Val	Lys
35	(2)	INF	TAMSO	KOI.	FOR S	SEO '	in w	n. 21	, .							
4D	, - ,		(i) S (A (B	EQUE L) LEI L) TY	NCE (NGTH: FE: a	CHARA 464 amino	ACTER ami	RISTI ino a	CS:	:						
				ECULI												
45 .	Met -32			UENCI Asn \			зу т					ly L	.y's ⊅ 20	rg L	ys V	'al
	Туг	Leu -15	Leu :	Ser 1	eu L	eu L	∈ບ I 10	le G	ly P	he T		sp C -5	ys V	al T	hr C	:y·s
50	His 1	GJ Y	Ser 1	Pro V	al A 5	sp I	le C	ys Ti		را 10	ys P:	ro A	ig A		1e P 15	10

	Met Asn Pro Met Cys Ile Tyr Arg Ser Pro Glu Lys Lys Ala Th 20 25 30	r Glu
5	Asp Glu Gly Ser Glu Gln Lys Ile Pro Glu Ala Thr Asn Arg Arg 35 40 45	
	Trp Glu Leu Ser Lys Ala Asn Ser Arg Phe Ala Thr Thr Phe Tys 50 55 60	
10	His Leu Ala Asp Ser Lys Asn Asp Asn Asp Asn Ile Phe Leu Ser 65 70 75	0.8
	Leu Ser Ile Ser Thr Ala Phe Ala Met Thr Lys Leu Gly Ala Cys 85 90 95	•
15	Asp Thr Leu Gln Gln Leu Met Glu Val Phe Lys Phe Asp Thr Ile 100 105 110	
	Glu Lys Thr Ser Asp Gln Ile His Phe Phe Ala Gln Leu Asn 115 120 125	
20	Arg Leu Tyr Arg Lys Ala Asn Lys Ser Ser Lys Leu Val Ser Ala 130 135 140 Arg Leu Phe Gly Asp Lys Ser Lou The Division of the Ser Lys Leu Val Ser Ala	
	Arg Leu Phe Gly Asp Lys Ser Leu Thr Phe Asn Glu Thr Tyr Gln 145 150 155 Ile Ser Glu Leu Val Tyr Gly Ala Lys Leu Gln Pro Leu Asp Phe	160
25	Glu Asn Ala Glu Gln Ser Arg Ala Ala Ile Asn Lys Trp Val Ser	
	Lys Thr Glu Gly Arg Ile Thr Asp Val Ile Pro Ser Clubbara	
30	Glu Leu Thr Val Leu Val Leu Val Asn Thr Ile Tyr Phe Lys Gly	
25	Trp Lys Ser Lys Phe Ser Pro Glu Asn Thr Arg Lys Glu Leu Phe 225	Tyr
35	Lys Ala Asp Gly Glu Ser Cys Ser Ala Ser Met Met Tyr Gln Glu (245 250 250	240 31y
40	Lys Phe Arg Tyr Arg Arg Val Ala Glu Gly Thr Gln Val Leu Glu I 260 265 270	eu
	Pro Phe Lys Gly Asp Asp Ile Thr Met Val Leu Ile Leu Pro Lys P 275 280 285	
45	Glu Lys Ser Leu Ala Lys Val Glu Lys Glu Leu Thr Pro Glu Val L 290 295 300	
		20
5 <i>0</i>	Pro Arg Phe Arg Ile Glu Asp Gly Phe Ser Leu Lys Glu Gln Leu Gl 325 330 335	
	Asp Met Gly Leu Val Asp Leu Phe Ser Pro Glu Lys Ser Lys Leu Pr 340 345 350	10

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	e 1	y 15	e Va 35		e Gl	u Gl	y Ar	9 As 36		sp Le	υ Τ	r Va	1 Se 36		p Al	a Phe
5	Hi.	s Ly 37		a Ph	e Le	u Gl	υ Va 37		n Gl	υ Gl	u Gl	у Sa 38		u Al	a Al	a Ala
	Se:		r Al	ā Vā) Va	1 II 35		e Pr	o Ar	g Se	r Le 39		n Pi	0 A.S	n Ar	g Val 400
10	Th	r Pho	≞ Ly:	s Al	a As 40		g Pr	o Ph	e Le	บ Va 41		e Il	e Ar	g G1	u Va. 41	l Pro 5
	Lev	As:	Th:	11 42 42 42 42 42 42 42 42 42 42 42 42 42		e Ph	e Mei	t Gly	y Ar 42	_	נא ו	a As	n Pro	6 Cy:		l Lys
15	(2)	INE	FORMA	TIOI.	v F0;	R 55(Q I Q	но:	28:							
20		(ii	((A) I (B) T (D) T	JENCI LENGI TYPE: TOPOI JLE I	rH: 4 : ami LOGY:	64 a no a lir	mind cid ear	ac:							
		(xi) SE	QUEN	CE I	ESCF	RIPTI	ON:	SEQ	ID N	0: 2	28:				
25	Met -32		Ser -30		Val	Ile	Gly	Thr -25		Thr	Ser	. ej?	-20	_	lys	val
	Tyr	Leu -15		Ser	Leu	Leu	Leu -10		Gly	Phe	Trp	Asp -5	_	Va1	Thr	Cys
30	His 1	Gly	Ser	Pro	Val 5		Ile	Cys	Thr	Ala 10		Pro	Arg	Asp	Ile 15	Pro
	Met	Asn	Pro	Меt 20		lle	Tyr	Arg	Ser 25		Glu	Lys	Lys	Ala 30		G1 u
35	Asp	Glu	Gly 35	Ser	Glu	Gln	Lys	Ile 40	Pro	Glu	A.la	Thx	Asn 45	Arg	Arg	Val
	Trp	Glu 50	Leu	Ser	Lys	Ala	Asn 55	Ser	Arg	Phe	Ala	Thr 60	Thr	Phe	Tyr	Gla
40	His 65	Leu	Ala	Asp	Ser	Ly's 70	Asn	Asp	Asn	Asp	75,	lle	Phe	Leu	Ser	Pro 80
	Leu	Ser	Ile	Ser	Thr 85	Ala	Phe	Ala	Met	Thr 90	Lys	Leu	e17.	Ala	Cys 35	Asn
45	Asp	Thr.	Leu	Gln 100	Gln	Leu	Met	Glu	Val 105	Phe	Lys	Phe	Asp	Thr 110	Ile	Ser
	Glu	Lys	Thr 115	Ser	Asp	Gln	Ile	His 120	Phe	Phe	Phe	Ala	Gln 125	Leu	Asn	Cys
50	Arg	Leu 130	Tyr	Arg	Lys	Ala	Asn 135	Lys	Ser	Ser	Lys	Leu 140	Val	Ser	Ala	Asn

											100					Asp 160
5	Ile	Ser	Glu	Leu	Val 165	Туг	Gly	Ala	Lys	Leu 170	Gln	Pro	Leu	Asp	Phe 175	Lys
	Glu	Asn	Ala	Glu 180	Gln	Ser	Arg	Ala	Ala 185	Ile	Asn	Lys	Trp	Val 190	Ser	Asn
10	Lys	Thr	Glu 195	G1 y	Arg	Ile	Thr	Asp 200	Val	Ile	Pro	Ser	Gl u 205	Ala	Ile	Asn
	Glu	Leu 210	Thr	Val	Leu	Val	Leu 215	Val	Asn	Thr	Ile	Tyr 220	Phe	Lys	Gly	Leu
15	Trp 225	Lys	Ser	Lys	Phe	Ser 230	Pro	Glu	Asn	Thr	Arg 235	Lys	Glu	Leu	Phe	Tyr 240
	Lys	Ala	Asp (Gly (Glu . 245	Ser	Cys	Ser	Ala	Ser 250	Met	Met	Туг	Gln	Glu 255	Gly
20	Lys	Phe .	Arg :	ryr) 260	Arg)	Arg	Val	Ala	Glu 265	Gly :	Thr .	Gln		Leu 270	Glu	Leu
	Pro						,	200					285			
25	Glu :					•						300				
	Gln (305				_					J	12					320
30	Pro A								•	30				3	335	
	Asp M							-	,43				3	50		
35	Gly I							00				3	65			
						_	, ,				31	80				
40	Ser T)					•				39	5				4	00
	Thr Pl				_				4.	U				43	5	
45	.Leu As	in Th	r Il: 42	e Ilo	e Ph	e Me	t Gl	у А: 42	g Va 25	i) Al	a As	n Pr	o Cy 43	vs Va	l L	ys .
	(2) IN															
50		1	SEQU (A) 1 (B) T (D) T	ENGT YPE:	H: 4' ami	64 a	amino acid	STIC Pac	S: ids							

(ii) MOLECULE TYPE: protein

		(×	i) S	EQUE	HCE	DESC	F:1 PT	3 01:	SEQ	2.0	HO:	29:				
5	Ме - 3		r Se -3	r Asi O	n Va	7 11	e G1	y 17t. -2	r Va S	l Tn	r Se	r Gl	у <u>Г</u> у -2		g Ly	s Va
	Ty	r Le -l	υ Leo S	u Se:	. Le	u Le	u Let	u Il 0	e G1	y Ph	e Ts	o As		s Va	1 Th	r C)
70		5 Gl;	y Se:	r Pro) Asj 5 .	o Ile	e C7:	s Th	r Ala 10		s Fr	o Ar	g As	p Il	
	Met	: Ası	n Pro	9 Met 20		5 116	ניצׄד ≘	r Arq) Se:		Glu	ı Ly:	s Ly:	5 Ala 30		r Gl
75	Asp	G1:	ر61 د 35	y Ser	Gli	Glr C	n Lys	5 Ile 40		o Glu	Ala	T'n:	7.51 45		g Ar	g Va.
	Trp	G1: 50) Ser	Lys	. Ala	Asn 55		: Arg	Phe	Ala	Th:		Phe	± Туз	c Gji
20	His 65		, Ala	Asp	Ser	Lys 70		Asp	Asn	Asp	Asn 75		Phe	: Lei	Ser	Pro 50
	Leu	Ser	: 11e	Ser	Thr 85	Ala	Phe	Ala	Met	Thr 90		Leu	Gly	Ala	Cys 95	
25	Asp	Thr	Leu	Gln 100		Leu	Мет	G) n	Val 105		Lyε	Phe	Asp	Thr 110		Ser
	Gju	Lys	Thr 115	Ser	Asp	Gln	Ile	His 120		Phe	Phe	Ala	Gln 125		Asn	Cys
30	Arg	Leu 130		Arg	Lys	Ala.	Asn 135	Lys	Ser	Ser	Lys	Leu 140	Val	Ser	Ala	Asn
	Arg 145	Leu	Phe	Gly	Аsp	Lys 150	Ser	Leu	Thr	Phe	Asn 255	Glu	Thr	Туг	Gln	Asp 160
35	Ile	Ser	Glu	Leu	Val 165	Tyr	Gly	Ala	1ys	Leu 170	GJn	Pro	Leu	Asp	Phe 175	Lys
	Glu	Asn	Ala	61u 180	Gln	Ser	Arg	Ala	Ala 185	lle	Asn	Lys	Trp	Val 190	Ser	Asn
40 .	Lys	Thr	Glu 195	Gly	Arg	lle	Thr	Asp 200	Val	lle	Pro	Ser	Gl u 205	Ala	.Ile	Asn
	Glu	Leu 210	Thr	Vàl	Leu	Val	Leu 215	Val	Asn	Thr	Ile	Туг 220	Phe	Lys	Gly	Leu
4E	Trp 225	Lys	5er	Lys	Phe	Ser 230	Pro	Glu	Asn	Thr	Arg 235	Lys	Gl v	Leu	Phe	Туг 240
45					245					250					255	
				Tyr . 260					265					270		
50	Pro	Phe	Lys 275	GJA '	Asp	Asp		Thr ! 280	Met	Val	Leu :		Le ນ 285	Pro	Lys	Pro

	Glu Lys Ser Leu Ala Lys Val Glu Lys Glu Leu Thr Pro Glu Val Leu 290 295 300
5	Gln Glu Trp Leu Asp Glu Leu Glu Glu Met Met Leu Val Val His Met 305 310 315 320
	Pro Arg Phe Arg Ile Glu Asp Gly Phe Ser Leu Lys Glu Gln Leu Gln 325 330 335
10	Asp Met Gly Leu Val Asp Leu Phe Ser Pro Glu Lys Ser Lys Leu Pro 340 345 350
	Gly Ile Val Ala Glu Gly Arg Asp Asp Leu Tyr Val Ser Asp Ala Phe 355 360 365
75	His Lys Ala Phe Leu Glu Val Asn Glu Glu Gly Ser Glu Ala Ala Ala 370 375 380
	Ser Thr Ala Val Val Ala Leu Gly Arg Ser Leu Asn Pro Asn Arg Val 385 390 395 400
20	Thr Phe Lys Ala Asn Arg Pro Phe Leu Val Phe Ile Arg Glu Val Pro 405 410 415
	Leu Asn Thr Ile Ile Phe Met Gly Arg Val Ala Asn Pro Cys Val Lys 420 425 430
25	(2) INFORMATION FOR SEQ ID NO: 30:
<i>30</i>	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 464 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear
	(ii) MOLECULE TYPE: protein (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:
35	Met Tyr Ser Asn Val Ile Gly Thr Val Thr Ser Gly Lys Arg Lys Val -32 -30 -25 -20
	Tyr Leu Leu Ser Leu Leu Leu Ile Gly Phe Trp Asp Cys Val Thr Cys
40	His Gly Ser Pro Val Asp Ile Cys Thr Ala Lys Pro Arg Asp Ile Pro 1 5 10 : 15
	Met Asn Pro Met Cys Ile Tyr Arg Ser Pro Glu Lys Lys Ala Thr Glu 20 25 30
45	Asp Glu Gly Ser Glu Gln Lys Ile Pro Glu Ala Thr Asn Arg Arg Val 35 40 45
	Trp Glu Leu Ser Lys Ala Asn Ser Arg Phe Ala Thr Thr Phe Tyr Gln 50 55 60
50	His Leu Ala Asp Ser Lys Asn Asp Asn Asp Asn Ile Phe Leu Ser Pro 65 70 75 80

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CP4 3D S (ii) (1, 1, 2, 3) , contribution a (1, 2, 3)

	Leu	Ser	11e	Ser	Th <i>r</i> 65	Аla	Phe	Ala	Met	Thr 90	Lys	Leu	Gl y	Ala	Cys 95	Asn
	Asp	Thr	Leu	700 67 u	G) rı	Lev	Met	G] u	Va) 105	Phe	Lys	Phe	Аsp	Thr 110	lie	Ser
5	Glu	Ъуѕ	Thr 115	5e r	Аsp	Gln	1)∈	His 120	Phe	Ph∈	Phe	Ala	Lys 125	Leu	Asn	Cys
	Arg	Leu 130	Tyr	Gln	Asn	Ala	Asn 135	Lys	5er	Ser	μλε	Ъеч 140	Věl	Ser	Ala	Asn
10	Arg 145	Leu	Phe	G) À	Asp	Lys 150	Ser	Leu	The	Phe	Asn 155	Glu	Thr	Tyr	Gln	Asp 160
	Ile	Ser	Glu	Leu	۷al 165	Tyr	G] y	A.) a	ГЛS	Leu 170	Gln	Pro	Leu	Asp	Phe 175	Ľ7.2
15				180					185				•	190	Ser	
			195					200					205		Ile	
20	Glu	Leu 210	Thr	Val	Leu	Val	Leu 215	Val	Asn	Thr	lle	Ту: 220	Phe	Lys	GJ A	Leu
•	225					230					235				Phe	240
25	_				245					250					Glu 255	
	_			260					265					270	Glu	
30			275					280					285		Lys	
		290					295					300			Val	
35	305					310					315				His	320
		_			325					330					Leu 335	
40				340					345					350	Leu	
			355					360					365		Ala	
45		370					375					380			Ala	
	385					350					395				Arg	400
50	Thr	Phe	Lys	Ala	A5n 405	Аrg	Pro	Phe	Leu	Val 410	Phe	Ile	Arg	Glu	Val 415	Pro

	Leu Asn Thr Ile Ile Phe Met Gly Arg Val Ala Asn Pro Cys Val Lys 420 425 430
5	(2) INFORMATION FOR SEQ ID NO: 31:
10	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 464 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: protein
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:
15	Met Tyr Ser Asn Val Ile Gly Thr Val Thr Ser Gly Lys Arg Lys Val -32 -30 -25 -20
	Tyr Leu Leu Ser Leu Leu Leu Ile Gly Phe Trp Asp Cys Val Thr Cys -15 -10 -5
20	His Gly Ser Pro Val Asp Ile Cys Thr Ala Lys Pro Arg Asp Ile Pro 1 5 10 15
	Met Asn Pro Met Cys Ile Tyr Arg Ser Pro Glu Lys Lys Ala Thr Glu 20 25 30
25	Asp Glu Gly Ser Glu Gln Lys Ile Pro Glu Ala Thr Asn Arg Arg Val 35 40 45
	Trp Glu Leu Ser Lys Ala Asn Ser Arg Phe Ala Thr Thr Phe Tyr Gln 50 60
30	His Leu Ala Asp Ser Lys Asn Asp Asn Asp Asn Ile Phe Leu Ser Pro 65 70 75 80
	Leu Ser Ile Ser Thr Ala Phe Ala Met Thr Lys Leu Gly Ala Cys Asn 85 90 95
3 5	Asp Thr Leu Gln Gln Leu Met Glu Val Phe Lys Phe Asp Thr Ile Ser 100 105 110
	Glu Lys Thr Ser Asp Gln Ile His Phe Phe Ala Lys Leu Asn Cys 115 120 125
40	Arg Leu Tyr Gln Asn Ala Asn Lys Ser Ser Lys Leu Val Ser Ala Asn 130 135 140
	Arg Leu Phe Gly Asp Lys Ser Leu Thr Phe Asn Glu Thr Tyr Gln Asp 145 150 155 160
45	Ile Ser Glu Leu Val Tyr Gly Ala Lys Leu Gla Pro Leu Asp Phe Lys 165 170 175
	Glu Asn Ala Glu Gln Ser Arg Ala Ala Ile Asn Lys Trp Val Ser Asn 180 185 190
50	Lys Thr Glu Gly Arg Ile Thr Asp Val Ile Pro Ser Glu Ala Ile Asn 195 200 205
	Glu Leu Thr Val Leu Val Leu Val Asn Thr Ile Tyr Phe Lys Gly Leu 210 215 220

	Trp 225		5er	Ъуs	Phe	Ser 230		· Glu	AS A	Thr	Arg 235		: Glu	ı Leu	?he	Tyr 240
5	ГÀг	: Ala	Asp	G1 y	Gl u 245		Cys	Ser	Ala	Ser 250		Met	Туг	G1:	Gl u 255	Gly
	Lys	Phe	Arg	Tyr 260		Arg	Val	Ala	G1 u 265		Thr	Gln	Val	Leu 270		Leu
10	Pro	Phe	Lys 275		Љsр	Asp	Ile	Thr 280		Val	Leu	11e	Leu 285		Lys	Pro
	Glu	290 290	Ser	Leu	ÆДа	Lys	Va <u>1</u> 295	Glu	Lys	Glu	Leu	Thr 300		Glu	Val	Leu
15	Gln 305		Trp	Leu	Asp	Glu 310	Leu	Glu	Glu	Met	Met 315	Leu	Val	Val	His	Met 320
	Pro	Arg	Phe	Arg	11e 325	Glu	Asp	GJ Y	Phe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	GŢυ
20	Аsp	Met	Gly	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	G1 v	Lys	Ser	Lys 350	Leu	Pro
	Gly	Ile	Val 355	Ala	Glu	Gly	Arg	Asp 360	Asp	Leu	Tyr	Val	Ser 365	Asp	Ala	Phe
25	His	Lys 370	Ala	Phe	Leu	G) u	Val 375	Asn	Glu	Glu	GJ Å	Ser 380	Glu	Ala	Ala	Ala
	Ser 385	Thr	Ala	Val		Ile 3 _, 90	Phe	Pro	Arg	Ser	Leu 395	Asn	Pro	Asn	Arg	Val 400
30	Thr	Phe	Lys	Ala	Asn 405	.e.rg	Pro	Phe	Leu	Val 410	Phe	Ile	Arg	Glu	Val 415	Pro
	Leu	Asn	Thr	11e 420	Ile	Phe :	Met	Gly	Arg 425	Val	Ala	Asn	Pro	Cys 430	Va]	Lys
35	(2)	INFO	RMAT	ION	FOR:	SEQ :	ID N	o: 3	2:						•	
40		((A (B) LE	NCE (NGTH PE: a	: 46	am ac	ino id		5						
					E TYI				FO 11	n No	. 39					
45	Met -32	Tyr :			•		51 y 1					31 y 1	Lys . -20	Arg :	Lys '	Vel
	Tyr	Leu : -15	Leu S	Ser 1	Leu I		Leu : ∙10	ile (2 1 Å 1	Phe 1	Trp)	Asp (Cys '	Val :	Thr (Cys
50	His	Gly s	Ser i	Pro \	/al # 5	sp I	1e (ys 1	Chr 1	10 1	ys E	ro A	Arg)	Asp 1	le ! 15	Pro

	м	et /	Asn	Pro	Мет 20	: Cy	s Il	e T	уr	Arg	5e 2	r Pr 5	° G]	u Ly	ys L		la T:	ır Glu
5	A	sp (lu	G1 y 35	Se	Gl:	u Gl	n L	ys	11∈ 40	Pro	o Gl	u Al	a Ti		sn A:	rg As	g Val
	T.	rp G	1 u 50	Leu	Ser	Ly	s Al	а А.	s n 5 5	Ser	Arq	g Ph	e Al		r Th	or Pi	эе Ту	r Gln
10	H:	is 1 55	eu .	Ala	Asp	Sei	- Ly 7	s A: 0	sn i	Asp	λsı	a As	p As 7	n Il 5	e Ph	e Le	u Se	r Pro 80
	Le	eu S	er :	Ile	Ser	Th:	Al	a Pì	ne A	Ala	Met	Th.	r Ly	s Le	บ Gl	y Al		s Asn 5
15	As	PT	hr 1	Leu	Gl n 100	Gln	Le	u Me	t (31 u	Val 105	Phe	≞ Ly:	s Ph	e As	p Th		e Ser
	G1	u L	ys 1	Thr 115	Ser	Asp	Gli	n I1	e 1	is 20	Phe	Phe	∍ Phe	≥ Ala	a Ly 12	s Le 5	u As:	n Cys
20		•	, ,					13	5					140)			a Asn
		_					130						155	•				1 Asp 160
25						103						170	•				175	
					100						182					190	0	Asn
30			-	,,					2	00				•	205	•		: Asn
	•		•					213	•					220				Leu
35							230						235				Phe	240
					•	243						250					Glu 255	-
40					00					2	65					270	Glu	
			٠.	,					28	U					285	-	Lys	
45								293						300			Val	
						-	310						315				His	320
50					٠	23					د.	30					Leu 335	
	Asp	Met	Gly	/ Le	0 V	al A	sp !	Leu	Ph∈	9 Se	er P	ro (Glu 1	Lys :		Lys 350	Leu	Pro

	61 Y	Ile	Val 355		G] u	677.	A.59	Asp 360		Lei	Tyr	Val	3er 365	Asp	Ala	Pho
s	His	Lys 370		Phe:	Let	61 u	Val 375		63 u	Glu	67.7	Ser		. Ala	Ala	A1
	5er 385		A.l a	Val	Va]	390 31e		Pro	Arg	Ser	395		Pro	Asn	Arg	Va:
10	Thr	Phe	Lys	Ala	Asn 405	Arg	Pro	Phe	Fen	V±3 430	Phe	Ile	Arg	Glu	Val 415	Fre
	Leu	Asn	Thr	11e 420	lle	Phe	Met	Gly	Arg 425		Ala	Asn	Pro	Cys 430	Vāl	Lys
15	(2)	INF														
20			() ()	A) L: B) T: D) T:	engti YPE: OPOL	d: 40 ami: DGY:	RACT: 64 au no au line pres	mino cid ear								
									SEQ :	ID N	o: 3	3:				
25	Met -32	Tyr	Ser -30	Asn	Val	Ile	ej À	Thr -25	Val	Thr	Ser	Gl y	Lys -20	Arg	Ly:s	Val
	Tyr	Leu -15	Leu	Ser	Leu	Leu	Leu -10	Ile	Gly	Phe	Trp	Asp -5	Cys	Val	Thr	Cys
30	1				5					10	Lys				15	
				20					25		ĞĮu			30		
·35	•		35					40			Ala		45			
		50					55				Ala	60				
40 .	65					70					Asn 75					80
					85					90	Lys		•		95	
<i>4</i> 5				100					105		Lys			110		
			115					120			Phe		125			
50		130					135				Ly's	140				
	Arg 145	Leu	Phe	Gly	Asp	Lys 150	Ser	Leu	Thr	Fhe	Asn 155	Glu	Thr	ፓ ሃ:ደ	Gln	Asp 160

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	Ile Ser Glu Leu Val Tyr Gly Ala Lys Leu Gln Pro Leu Asp Phe Lys 165 170 175
5	Glu Asn Ala Glu Gln Ser Arg Ala Ala Ile Asn Lys Trp Val Ser Asn 180 185 190
	Lys Thr Glu Gly Arg Ile Thr Asp Val Ile Pro Ser Glu Ala Ile Asn 195 200 205
10	Glu Leu Thr Val Leu Val Leu Val Asn Thr Ile Tyr Phe Lys Gly Leu 210 215 220
	Trp Lys Ser Lys Phe Ser Pro Glu Asn Thr Arg Lys Glu Leu Phe Tyr 225 230 235 240
15	Lys Ala Asp Gly Glu Ser Cys Ser Ala Ser Met Met Tyr Gln Glu Gly 245 250 255
	Lys Phe Arg Tyr Arg Arg Val Ala Glu Gly Thr Gln Val Leu Glu Leu 260 265 270
20	Pro Phe Lys Gly Asp Asp Ile Thr Met Val Leu Ile Leu Pro Lys Pro 275 280 285
	Glu Lys Ser Leu Ala Lys Val Glu Lys Glu Leu Thr Pro Glu Val Leu 290 295 300
25	Gln Glu Trp Leu Asp Glu Leu Glu Glu Met Met Leu Val Val His Met 305 310 315 320
	Pro Arg Phe Arg Ile Glu Asp Gly Phe Ser Leu Lys Glu Gln Leu Gln 325 330 335
30	Asp Met Gly Leu Val Asp Leu Phe Ser Pro Glu Lys Ser Lys Leu Pro 340 345 350
	Gly Ile Val Ala Glu Gly Arg Asp Asp Leu Tyr Val Ser Asp Ala Phe 355 360 365
35	His Lys Ala Phe Leu Glu Val Asn Glu Glu Gly Ser Glu Ala Ala Ala 370 375 380
	Ser Thr Ala Val Val Ala Leu Gly Arg Ser Leu Asn Pro Asn Arg Val 385 390 395 400
40	Thr Phe Lys Ala Asn Arg Pro Phe Leu Val Phe Ile Arg Glu Val Pro 405 410 415
	Leu Asn Thr Ile Ile Phe Met Gly Arg Val Ala Asn Pro Cys Val Lys 420 425 430
45	(2) INFORMATION FOR SEQ 1D NO: 34:
	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 464 amino acids
50	(B) TYPE: amino acid (D) TOPOLOGY: linear
	(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34: Met Tyr Ser Asn Val 11e Gly Thr Val Thr Ser Gly Lys Arg Lys Val -32 -30 -25 Tyr Leu Leu Ser Leu Leu Leu lle Gly Phe Trp Asp Cys Val Thr Cys His Gly Ser Pro Val Asp lle Cys Thr Ala Lys Pro Arg Asp Ile Pro 1 15 70 Met Asn Pro Met Cys Ile Tyr Arg Ser Pro Glu Lys Lys Ala Thr Glu 20 25 30 Asp Glu Gly Ser Glu Gln Lys Ile Pro Giu Ala Thr Asn Arg Arg Val 35Trp Glu Leu Ser Lys Ala Asn Ser Arg Phe Ala Thr Thr Phe Tyr Gln 50 60 His Leu Ala Asp Ser Lys Asn Asp Asn Asp Asn Ile Phe Leu Ser Pro 65 70 80 Leu Ser Ile Ser Thr Ala Phe Ala Met Thr Lys Leu Gly Ala Cys Asn 85 90 95 Asp Thr Leu Gln Gln Leu Met Glu Val Phe Lys Phe Asp Thr Ile Ser Glu Lys Thr Ser Asp Gin Ile His Phe Phe Phe Ala Lys Leu Asn Cys Arg Leu Tyr Gln Lys Ala Asn Lys Ser Ser Lys Leu Val Ser Ala Asn 130 135 Arg Leu Phe Gly Asp Lys Ser Leu Thr Phe Asn Glu Thr Tyr Gln Asp 145 150 150 30 Ile Ser Glu Leu Val Tyr Gly Ala Lys Leu Gln Pro Leu Asp Phe Lys Glu Asn Ala Glu Gln Ser Arg Ala Ala Ile Asn Lys Trp Val Ser Asn 180 185 190 35 Lys Thr Glu Gly Arg Ile Thr Asp Val Ile Pro Ser Glu Ala Ile Asn 195 200 205Glu Leu Thr Val Leu Val Leu Val Asn Thr Ile Tyr Phe Lys Gly Leu 210 215 220 40 Trp Lys Ser Lys Phe Ser Pro Glu Asn Thr Arg Lys Glu Leu Phe Tyr 225 230 . 235 Lys Ala Asp Gly Glu Ser Cys Ser Ala Ser Met Met Tyr Gln Gly Gly 245 250 45 Lys Phe Arg Tyr Arg Arg Val Ala Glu Gly Thr Gln Val Leu Glu Leu 260 265 270 Pro Phe Lys Gly Asp Asp Ile Thr Met Val Leu Ile Leu Pro Lys Pro 275 280 285 50

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Glu Lys Ser Leu Ala Lys Val Glu Lys Glu Leu Thr Pro Glu Val Leu

		290					295	,				360				
5	Gln 305	Glu	Trp	Leu	Asp	Glu 310	Leu	Glu	Glu	Met	Мес 315	Leu	Val	Va]	His	. Ме 32
	Pro	Arg	Phe	Arg	Ile 325	Glu	Asp	Gly	Phe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	
10	Asp	Met	Gly	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	Glu	Lys ·	Ser	Lys 350		Pr
		Ile	555					200					365			
15		Lys 370					3,3					360				
		Thr				330					395					400
20		Phe			703					410					415	
	Leu	Asn '	Thr :	11e 420	Ile	Phe	Met	Gly	Arç 425	Val	Ala	Asn	Pro	Cys 430	Val	Lys
25	(2)	INFO	i) SI (A)	EQUE	NCE NGTH	CHAR : 46	ACTE 4 am	RIST ino		s						
30		(ii) (xi)	MO'TE	TO	POLO E TY		line. Prot	ar ein	FO 7.1	n No.	. 25.					
25	Met T -32 Tyr I	Tyr S -	er A	.sn ∖	al 1	lle (Leu I	31y 1	Thr \ -25	√al 7	hr s	Ser (51y 1 -	·20 :ys \			
o	His G 1 Met A				3	sp I	le C			10		ro A	rg A		15	
	Met A			٠,					25	·· · ·	•		•	30·.	٠.	•
5	Trp G	lu Le	_					40					45			
	His Le						33				•	50				
0	65 Leu Se			r Th	r Al	, 0			et Th	r Ly	15					80
				9	5				9	0					95	

	Asp	The	Leu	100		Lei	net -	: G)	u Va. 10		e Ly:	s Ph	a As	p Th.		∈ Ser
	G1 u	Lys	Thr 115		Asp	G1 r	ıle	Hi:		e Phe	e Phe	= Als	125		AS1	n Cys
5	Arg	leu 130	-	Arg	Asn	٨Į ē	Asn 135		s Se	r Se:	: Lys	le:		l Sei	r 7-1:	a Asn
	Arg 145		Phe	GJ Y	Asp	Lys 150		Lei	The		45r		Th:	Ty:	: Gl:	7 Asp 160
10	lle	Ser	Gl u	Leu	Val 165	Tyr	Gly	.Ala	r Lys	170		Pro	Lev	ı Asp	Phe 175	Lys
	GŢη	Asn	Ala	Glu 180	G1 is	Ser	Arg	Ala	185		Asn	Lys	Trp	Val 190		. Asn
15	Lys	Thr	Glu 195	63 y	Arg	Ile	Thr	Asp 200		Ile	Pro	5er	G1 u 205		Ile	Asn.
	Glu	Leu 210	Thr	Val	Leu	Val	Leu 215	Val	Asn	Thr	Ile	ту:r 220		Lys	G) y	Leu
20	Trp 225	Lys	Ser	Lys	Phe	Ser 230	Pro	Glu	Asn	Thr	Arg 235		Glu	ั⊤≅ก	Phe	Tyr 240
	Lys	Αla	Ąsp	Gl y	Gl u 245	Ser	Cys	Ser	Ala	Ser 250	Мет	Мет	Tyr	Gln	Glu 255	Gly
25	Lys	Phe	Arg	Tyr 260	Arg	Arg	Val	Ala	Glu 265	G) y	Thr	Gln	Val	Leu 270	Glu	Leu
	Pro	Phe	Lys 275	с 1 у	Asp	Asp	Ile	Thr 280	Met	Val	Leu	Ile	Leu 285	Pro	Lys	Pro
<i>30</i>	Glu	Lys 290	Ser	Leu	Ala	Lys	Val 295	Glu	Lys	Glu	Leu	Thr 300	Pro	Glu	Val	Leu
•	Gln 305	Glu	Trp	Leu	Asp	Glu 310	Leu	Glu	Glu	Met	Met 315	Leu	Val	Vāl	His	Met 320
35	Pro	Arg	Phe	Arg	Ile 325	Glu	A.sp	GJ 7.	Phe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	Gln
,•	Asp	Met	Gly	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	Glu	Lys	Ser	Lys 350	Leu	Pro
40 ·	Gly	Ile	Val 355	Ala	Glu	G1 y	Arg	Asp 360	Asp	Leu	Tyr	Val	Ser 365	A.sp	Ala	Phe
	His	Lys 370	Ala	Phe	Leu		Val 375	Asn	Glu	Glu	Gly	Ser 380	Gl u	A.la	Ala	Ala
<i>4</i> 5 .	5er 385	Thr	A.la	Val		11e 350	Phe	Pro	Arg	Ser	Leu 395	Asn	Pro	A.s.n	Arg	Val 400
					405					Val 410					415	
50	Leu .	Asn		11e 420	Ile	Phe !	Me I		Arg 425	Vāl.	Ala.	Asn		Cys 430	Val	Lys

(2) INFORMATION FOR SEQ ID NO: 36:

5				(A) (B)	LENG Type	E CH TH: : am LOGY	464 ino	amin acid	o ac	S: ids						
		(i:	i) M	OLEC	ULE	TYPE	: pr	otei	ח							
		(x:	i) S	EQUEI	NCE	DESC	RIPT	ION:	SEQ	ID	NO: 1	36:				
10	Me: -3:	t Ty: 2	r Se:	Ası	n Va	1 11	e Gl	y Th -2	r Va 5	1 Th.	r Se	r G1	y Ly:		g Ly	s Val
15	Ty	- Lev	ı Lei	ı Sei	r Le	u Lei	Let -10	ı Ile	e Gl	y Ph	e Trp	As ₁	p Cys	s Va	l Th	r Cys
	Hi:	5 Gly 1	/ Se:	Pro	Va.	l Asp 5	o Ile	e Cys	s Th	r Ala		Pro	o Arg	a Ası	P Ile 1	≥ Pro 5
20	Met	Asn	Pro) Met 20	: Су:)	s Ile	≘ Туг	: Ar	3 Se:	r Pro	o Glu	Lys	s Lys	Ala 30		r Glu
	Asp	Glu	Gly 35	Ser	Glu	Glr.	Lys	11e	e Pro	o Glu	Ala	Th	: Asr 45		g Arg	y Val
25	Trp	61u 50	Leu)	Ser	Lys	s Ala	Asn 55	Ser	Arç	g Phe	: Ala	Th:		Phe	туг	r Gln
20	His 65	Leu	Ala	Asp	Sea	Lys 70	Asn)	Asp	Ası	n Asp	Asn 75	Ile	Phe	: Le	Ser	Pro 80
30	Leu	Ser	Ile	Ser	Th:	Ala	Phe	A.1 a	Met	Th:		Leu	Gly	Ala	Cys 95	Asn
30	Asp	Thr	Leu	Gln 100	Gln	Leu	Met	Glu	Val 105	Phe	Lys	Phe	Asp	Thr		Ser
05	Glu	Lys	Thr 115	Ser	Asp	Gln	Ile	His 120	Phe	Phe	Phe	Ala	Lys 125	Leu	Asn	Cys
35	Arg	Leu 130	Tyr	Arg	Gln	Ala	Asn 135	Lys	Ser	Ser	Lys	Leu 140	Val	Ser	Ala	Asn
40	Arg 145	Leu	Phe	Gly	Asp	Lys 150	Ser	Leu	Thr	Phe	Asn 155	Glu	Thr	Tyr	Gln	Asp 160
40	Ile	Ser	Glu 	Leu	Val 165	Tyr	Gly	Ala	Lys	Leu 170	Gln	Pro	Leu	Asp	Phe 175	Lys
45	Glu	Asn	Ala	Glu 180	Gln	Ser	Arg	Ala	Ala 185	Ile			Trp			Asn
43	Lys	Thr	Glu 195	Gly	Arg	Ile	Thr	Asp 200	Val	Ile	Pro	Ser	Glu 205	Ala	Ile	Asn
50	Glu	Leu 210	Thr	Val	Leu	Val	Leu 215	Val	Asn	Thr	lle	Tyr 220	Phe	Lys	Gly	Leu
	Trp 225	Lys	Ser	Lys	Phe	Ser 230	Pro	Glu	Asn	Thr	Arg 235	Lys	Glu	Leu	Phe	Tyr 240

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and the entry letter in the artists of a

	Г'n	s Ala	. Asr	GJ Ž	G] u		Cys	Ser	Ala	ser 250		: !4e 1	ту	r Glr	61 to 255	Gly
s	Ly	s Phe	. Arg	Tyr 260		<i>F</i> .rg	Val	<i>1</i> .1 а	Glu 265		Thr	Glr	va)	l Leu 270		: Leu
	Pro	o Phe	1375 275	eĵ λ	Asp	Asp	Ilė	Thr 280		Vàl	Leu	Ile	: Let 285		Lys	Pro
10	Gli	190ء 290ء د		Leu	Ala	Lys	Val 295	Glu	Γλ.ε	Glu	Leu	Thr 300		Glu	Val	Leu
	Glr 305	Glu S	Trp	Leu	Аsp	G] ս 310	Leu	Glu	Glu	Met	Met 315	Leu	Val	Val	His	Met 320
15	Pro) Arg	Phe	Arg	11e 325	Glu	Аsp	G] y	Phe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	Gln
	Asp	Met	GJ Y	Leu 340	Val	Asp.	Leu	Phe	Ser 345	Pro	Glu	Lys	Ser	Lys 350	Leu	Pro
20	G 1 y	Ile	Val 355	Ala	Glu	Gly	Ærg	Asp 360	Asp	Leu	Tyr	Val	Ser 365	Asp	Ala	Phe
	His	Lys 370	Ala	Phe	Leu	Glu	Val 375	Asn	Glu	Glu	Gly	Ser 380	Glu	Ala	Ala	Ala
25	Ser 385	Thr	Ala	Val	Val	11e 390	Phe	Pro	Arg	Ser	Leu 395	Asn	Pro	Asn	Arg	Val 400
	Thr	Phe	Lys		Asn 405	Arg	Pro	Phe	Leu	Val 410	Phe	lle	Arg	Glu	Val 415	Pro
30	Leu	Asn		11e 420	Ile	Phe	Met	G] y	Arg 425	Val .	Ala	Asn	Pro	Cys 430	Val	Lys
35	(2)	INFC	i) S (A (B	EQUE	NCE (NGTH PE: i	CHAR : 46 amin	ACTE 4 am 0 ac	FIST ino id		s				٠		
40			MOL													
	Met - 32	Tyr	SEQI Ser <i>3</i> -30				Sly :					31 y :	Lys -20	Arg :	Lys '	Val
45	Туг	Leu :	Leu S	Ser 1	eu l		Leu 1 ·10	(le (Sly I	Phe I	rp A	-5	Cys	Val :	Chr (Cys
	His 1	Gly :	Ser F	, to /	al A 5	sp I	le C	ני פיע:	Thr A	1a 1	צינ.∃	ro)	Arg 2	Asp I	le I 15	Pro
50	Met	Asn i	M OI	let C 20	ys I	le T	'y <i>≖ ≯</i>	rg S	er P 25	ro G	lu I	ys 1	ys ?	4 <u>la</u> 7 30	hr G	lυ

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	A.s	p Gl	u G1 3	у 5е 5	r G1	u Gl	n Ly	s I) 4	e Pr O	o G1	u Al	a Th		n Ar 5	g Ar	g Val
5	Tr	p G1 5	0. n Fe	u Se	r Ly	s Al	a As	n 5e 5	: Ar	g Ph	e Al	a Th		r Ph	е Ту	r Gl
	Ні 6	s Le 5	u Al	a As	p Se	: Ly 7	s Asi O	n As	p As	n As	P Asi		e Ph	e Le	u Se.	r Pro
10					r Th: 85	>				9	D				9	5
				10					10	5				11	0	
15			11:	•	r Asp			120	Б				125	5		
		13(J		g Lys		135	•				140)			
20	.143	3			/ Asp	150)	•			155	•			•	160
					165)				170)				175	•
25				180					185	5				190)	
			195	•	Arg Leu			200	,				205	,		
30		210	,		Phe		215					220			_	
٠	225	,			Glu	230					235					240
35					245 Arg					250					255	
				260	Asp				265					270		
40			2/5		Ala			280					285			
	Gln	290			Asp		295					300				
15	303			•	Ile	310					315					320
				Leu	325 Val					330					335	
60			Val	340	Glu (345					350		
			355					360					365	•		-

		Нis	: Ъу: 37(a Ph	e Le	υ G]	υ Va 37		in G)	.u G)	u G)	y 5∈ 38		u Al	a Al	6 Ala
5		Ser 385		r Ai	a Va	l Va	39 1 11		e Fr	o Ar	ç Se	r Le 35		n Ps	o As	n Ar	g Val 400
	•	Thr	Phe	E Ly:	s #1	a As 40		g Fr	o Ph	e Le	υ Va 41		e Il	e Ar	9 G1	u Vā. 4]	1 Pro 5
10	1	Lev	Asr	Th:	r Il 42		e Ph	e Me	t Gl	y Ar 42		1 Ala	As:	n Pr	6 Cy:		l Lys
	1	(2)	IHF	MGO"	ATIO	N FOI	R SE	o id	NO:	38:							
15				(i)	SEQ1 (A) :	UENC! LENG! LYPE: LOPO!	CH:	4.P.A.C. 464 ino 4	reri. amin ecid	STIC							
20						JLE T		_					•				
20	м	et								-	ID 1. Thr			Lys	Arg	Lys	. Val
	-	32		-30					-25	5				-20)		C)·s
25			-15					-10)				~ 5				_
		15	G1 À	Ser	Pro	Val 5	Asp	Ile	. Cys	Thr	10		Pro	Arg	Asp	Ile 15	Pro
30		et	Asn	Pro	Met 20		Ile	Tyr	Arg	Ser 25		Glu	Lys	Lys	Ala 30	Thr	Glu
	<u>.</u> A.:	sp	Glu	G1 y 35	Şer	Glu	Gln	Lys	Ile 40		G1 u	Ala	Thr	.4.5 n	Arg	Arg	Vel
35	T	гр	Glu 50	Leu	Ser	Lys	Ala	Asn 55	Ser	Arg	Phe	Ala	Thr 60	Thr	Phe	Tyr	Gln
		is 55	Leu	Ala	Asp	Ser	Lys 70	Asn	Asp	.Asn	Asp	Asn 75	lle	Phe	Leu	Ser	Pro 80
40	Le	eu .	Ser	Ile	Ser	Thr 85	Ala	Phe	Ala	Met	Thr 90	Lys	Leu	Gly	Ala	Cys 95	Asn
	As	p '	Thr	Leu	Gln 100	Gln	.Leu	Met	Glu	Val 105	Phe	Lys	Phe	Asp	Thr 110	Ile	Ser
45	Gl	.ນ]	Lys	Thr 115	Ser	Asp	Gln	Ile	His 120	Phe	Phe	Phe	Ala	Lys 125	Leu	Asn	Cλε.
43	6 1	ת ב	Leu 130	Tyr	Arg	Lys	Ala	Asn 125	Lys	Ser	Ser	Lys	Leu 140	Va 1	Ser	Ala	Asn
	Ar 14	g I 5	Leu	Phe	Gly	Asp	Lys 150	Ser	Leu	Thr	Phe	Asn 155	Glu	Thr	Tyr	Gln	Asp 160
50	11	e 5	Ser	Glu	Leu	Val 165	Tyr	eīà	Ala	Lys	Leu 170	Gln .	Pro	Leu		Phe 175	Lys

	Glu	A.sn	Ala	G1 u 190	Glr) Ser	Arç	, A.1 a	Ala 185		. Asn	lys	Trp	Va!		: Asn
5	Lys	The	Gl u 195	Gl y	Arg	ı 11e	Thr	Asp 200	Val	Ile	e Pro	Ser	Glu 205		Ile	e Asn
	Glu	Leu 210	Thr	Val	Leu	Val	Leu 215	Val	Asn	The	Ile	Tyr 220		Lys	Gly	/ Leu
10	Trp 225	Lys	Ser	Lys	Phe	Ser 230	Pro	Glu	Asn	Thr	Arg 235		Glu	Leu	Phe	Tyr 240
	Σys	Ala	Asp	G1 ý	Glu 245	Ser	Cys	Ser	Ala	Ser 250		Met	Tyr	Gln	G1 u 255	Gly
15	Lys	Phe	Arg	Tyr 260	Arg	Arg	Val	Ala	Glu 265	Gly	Thr	Gln	Va <u>l</u>	Leu 270		Leu
	Pro	Phe	Lys 275	Gly	Asp	Asp	Ile	Thr 280	Met	Val	Leu	Ile	Leu 285	Pro	Lуs	Pro
20	Glu	Lys 290	Ser	Leu	Ala	Lys	Val 295	Glu	Lys	Glu	Leu	Thr 300		Glu	Val	Leu
	Gln 305	Glu	Trp	Leu	Asp	Glu 310	Leu	Glu	Glu	Met	Met 315	Leu	Val	Val	His	Met 320
25	Pro	Arg	Phe	Arg	Ile 325	Glu	Asp	elà	Phe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	
	Asp	Met	Gly	Leu 340	Va l	Asp	Leu	?he	Ser 345	Pro	Glu	Ljrs	Ser	Lys 350	Leu	Pro
30	Gly	Ile	Val 355	Ala	Glu	Gly	Arg	Asp 360	Asp	Leu	Tyr	Val	Ser 365	Asp	Ala	Phe
	His	Lys 370	Alá	Phe	Leu	Glu	Val 375	Asn	Glu	Glu	Gly	Ser 350	Glu	Ala	Ala	Ala
	Ser 385	Thr	Ala	Val	Val	Ile 390	Val	Pro	Arg	Ser	Leu 395	Asn	Pro	Asn	Arg	Val 400
	Thr	Phe	Lys .	Ala.	Asn 405	Arg	Pro	Phe		Val 410	Phe	Ile	Arg	Glu	Val 415	Pro
40	Leu .	Asn '	Thr	Ile : 420	Ile	Phe	Met		Arg 425	Val	Ala .	Asn	Pro	Cys 430	Val	Lys
. 45	(2)	INFO	RMAT	ION I	FOR .	SEQ :	ID N	O: 3	9:							
70		(3	(A)	LEN TYE	GTH E: a	CHAR : 46 amin GY: 1	am:	ino a id		5						
50		(ii)														
	((xi)	SEQU	ENCE	DES	CRI	10 I T	1: SE	Q 1 [) ИО:	39:					

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THE MAINING THE CONTINUES AND A

	Мез - 33		5e: -3(n Väl	1 1) 6	e G)	y Tr. -2		l Tr	r Se	r Gl	y Ly -2		g py	s Val
5	T';	- Leu		ı Sel	r Let	ı Let	- le		e 61	y Ph	e Tr	დ გაც 		s Val	1 Th	r Cys
	His		, 5ē1	Pro	val S		: 116	∈ Су	s Th	s Al		s Pr	o Ar	ç Ası	5]];];	e Pro 5
10	Met	: Asn	Pro	. Жет 20		. 11e	Tyr	e As	9 Se. 2:		5 G)	o Lys	Ly:	s Ala 30		r Glu
	Asp	, Glu	G1 y 35		: Glu	Gln	Lys	5 Ile		o Gl	: Ala	Th:	AS1		a Arq	ya)
15	Trp	Glu 50		Ser	Lys	Ala	Asr. 55		Az ç	g Phe	: Ä):	Thr EQ		Phe	туі	Gln
	His 65		Ala	Asp	Ser	Lys 70		. Asp) Asr) Asp	, Asr 75		Phe	Leu	ser	80
20	Leu	Ser	īle	Ser	Thr 85	Ala	Phe	.la	Мет	. Thr 90	_	Leu	Gl y	· Ala	Cys 95	Asn
				100					105	;	_		·	110		: Ser
25			115					120					125			Cys
		130			•		135				-	140				Asn
30	145					150	•	•			155			-		Asp 160
ı					165		,			170				-	175	Lys
35				180					185					190		A.sn
			195		Arg			200					205			
40		210			Leu		215					220		-		
	225				Phe Glu	230					235				·	240
45					245 Arg					250					255	•
				260	Asp.				265					270		
50			275		Ale :			280					285			
•		290	- -	-			295	- - -	, 			300		-, u		260

	G. 3	ln G 05	lu T	rp L	eu A	sp G	1 u 10	Leu	C) (u G1	u Me	t Me 31		u Va	s) Va	1 Ha	s Met 320
5	P.	ro A	rg Pi	he A	rg I. 3.	le G 25	lu	Asp	G1 y	y Pho	33	r Le O	u Ly	's G]	lu Gl	n Le 33	u Gln 5
	As	sp Me	et G	ly Le 34	eu V. 40	el A	sp	Leu	Phe	345	2:0	G [u Ly	's Se	r Ly 35		u Pro
10	G1	ly II	le Va 33	al Al 55	la G	lu G	1у.	Arg	Asp 360	Asp	Le	ту.	r Va	1 Se	r A.s	p Al	a ?he
	Hi	s Ly 37	/s Al	.a Pł	ne Le	eu G	lu '	Val 375	Аѕл	Glu	Glu	2 G1	y Se 38		u Al	ā Al	a Ala
15	Se 38	r Th	r Al	a Va	ıl Va	1 A	la 1 90	Leu	G1 y	Arg	Ser	1 Let 393	ı As	n P <i>z</i>	o As	n As	y Val 400
	Th	r Ph	e Ly	s Al	.a As 40	n A:	rg!	Pro	Phe	Leu	Val 410	Phe	: I1	e Ar	g G1	u Va 41	Pro
20	Le	u As	n Th	r I1 42	∈ Il 0	e Pi	ne M	Met	Gly	Arg 425	Val	Ala	As:	n Pr	o Cy 43		Lys
	(2) IN	FORM	A.TIO	N FO	R SE	Q I	D N	10:	40:							
25				(A) (B)	UENC LENG TYPE TOPO	TH: : am	4 64 11 no	l am	ino id	TICS aci	: ds						
					ULE '												
30	Me t - 32	ту		: Ası	NCE i			ly '						Lys -20		. Lys	Val
35	Туг	Let -15	ı Lev	ı Sei	Let	ı Le	u L			Gly	Phe	Trp	Asp -5			Thr	Cys
	His 1	G1 y	, Ser	Pro	Val 5	Ası	5 I.	le (:ys	Thr	Ala 10	Ile	Pro	Arg	Ser	11e 15	Pro
40	Met	Asn	Pro	Met 20	Cys	Ile	÷ Ty	yr A	irg	Ser 25	Pro	G1 u	Lys	Lys	Ala 30	Thr	Glu
	Asp	Glu	Gly 35	Ser	Glu	Glr	L)	ys I	1e 40	Pro	Glu	Alą	Thr	Asn 45	Arg	Arg	Val
45	Trp	Glu 50	Leu	Ser	Lys	Ala	As 5	sn S 55	er :	Arg	Phe	Ala	Th <i>z</i> 60	Thr	Phe	туг	Gln
	His 65	Leu	Ala	Asp	Ser	Lys 70	As	n A	sp /	Asn A	Asp.	Asn 75	lle	Phe	Leu	Ser	Pro 80
50					85						90					Cys 95	
	Asp	Thr	Leu	Gln 100	Gln	Leu	Ne	t G	lu V 1	/al F .05	he !	-y·s	Phe	Asp	Thr 110	Ile	Ser

	Glu	ı Pis	Th:		: Asp	· Glr	, 114	÷ Hi: 120		e Pho	e Ph	÷ 21.	5 Ly: 12:		. Ası	n Cys
5	Arg	130	-	: Arç	Lys	Ala	135	-	s Se.	s Ses	r Ly:	140		l Sei	: Ala	s Asn
	Arg 145		Phe	G1 y	Asp	150		Lei	Th:	r Phe	2 Asi 153		J Thi	туг	Glr	0 Asp 160
10	Ile	Ser	СЭ и	L÷u	Val 165		Gly	Ala	Lys	170		Pro) Leu) Asp	Phe 175	: Lys
	G1 n	Asr.	Äla	Gl u 180		Ser	Arg	Ala	Ala 185		: Asr	Lys	Trp	Val 190		Asn
15	ŗλ2	Thr	Gl u 195		Arg	Ile	Thr	Asp 200		Ile	Pro	Ser	61u 205		Ile	Asn
	Glu	Leu 210		Val	Leu	Val	Ն∈ս 215		Asn	Thr	Ile	Tyr 220		Lys	Gly	Ъеи
20	Trp 225		Ser	Lys	P'n∈	Ser 230	Pro	Glu	Asn	Th:r	Arg 235		Glu	Leu	Phe	Tyr 240
	Lуs	Ala	Asp	GГÀ	Glu 245	Ser	Cys	Ser	Ala	Ser 250	Met	Met	Tyr	Gln	Glu 255	
25	Lys	Phe	Arg	Tyr 260	Arg	Arg	Val	Ala	61 u 265		Thr	Gln	Va <u>l</u>	Leu 270	Glu	Leu
	Pro	Phe	Lys 275	Gly	Asp	ьsр	Ile	Thr 280	Met	Val	Leu	Ile	Leu 285	Pro	Lys	Pro
30	Glu	Lys 290	Ser	Leu	Ala	Lys	Val 295	Glu	Lys	Glu	Leu	Thr 300	Pro	Glu	Val	Leu
	Gln 305	Glu	Trp	Leu	Asp	Glu 310	Leu	Glu	Glu	Met	Met 315	Leu	Val	Val	His	Met 320
35	Pro	Arg	Phe	Arg	11e 325	Glu	Asp	Gly	Phe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	Gln
	Asp	Met	Gly	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	Glu	Lys	Ser	Lys 350	Leu	Pro
40	Gly	Ile	Val 355	Ala	Glu	Gly	Arg	Asp 360	Asp	Leu	Туг	Val	Ser 365	Asp	Ala	Phe
_	His	Lys 370	Ala	Phe	Leu	Glu	Val 375	Asn	Glu	Glu	еŢĀ	Ser 380	Glu	Ala	Ala	Ala
45	Ser 385	Thr	Al a	Val		Ile 390	Phe	Pro	Arg	Ser	Leu 395	Asn	Pro	Asn	Arg	Val 400
50	Thr	Phe	Ly s		Asn 405	Arg	Pro	Phe	Leu	Val 410	Phe	Ile	Arg	Glu	Val 415	Pro
-	Leu	Asn		11e 420	lle	Phe	Met	61 y	Arg 425	Val	Ala.	⊱s n		Cys 430	Val	Lys
		•														

	(2) II	1FOPJ	MATIC)N F	OR S	EQ I	יסא פ	: 41	:		•				
5			(i	(B)	TYP:	CE CI GTH: E: ar OLOGY	464 nino	ami: acio	no ad i	CS: cids						
				MOLEC SEQUE												
10	Μe	t Ty											_			
	-3	22	-3	30				-2	5	.1 11	ı. Se	r G1	.y Ly -2		g Ly	s Va
	Ty	r Le -1	u Le 5	u Se	r Le	u Le	u Le -1	u Il O	e Gl	y Ph	e Tr	p As -	р Су 5	s Va	l Th	r Cy
15	Hi	s G1 1	y Se	r Pr	o Va	1 As 5	p Il	e Cy	s Th	r Al l	a Il O	e Pr	o Ar	g Se	r Il 1	
	Me	t As:	n Pr	o Me 2	t Cy O	s Il	е Ту	r Ar	g Se 2	r Pr 5	o Gl	u Ly	s Ly	s Al.		r Gl
20		p Gl	3	,				41	U				4 :	5		
	Tr	p Gl: 50	ı Le	u Ses	r Ly	s Al	a Ası 5	n Se: S	r Are	g Ph	e Ala	a Th	r Th	r Phe	≘ Ту	c Glr
25	•					11	J				75	5				80
		ı Ser			3	3				9(י				9.5	,
30		Thr		100	' •				105	•				110)	
		l Lys	113	,				120					125			
35		Leu 130					135					140				•
						130					155					160
40		Ser								170					175	-
	•	Asn		100					182			•		190		
75		Thr	175					200					205			
		Leu 210					213					220				
o	Trp 225	Lys	Ser	Lys	Phe	Ser 230	Pro	Glu	Asn	Thr	Arg 235	Lys	Glu	Leu		Tyr 240

EP 0 568 833 A1 · ·

	Lys	Ala	Asp	G3 y	Glu 245	Ser	Cys	Ser	A)a	Ses 250		Ne:	Tyr	61n	61u 255	
5	Lys	Phe	Arg	Tyr 260	Arg	Arg	Val	7.1 a	61 u 265	61 y	Th:r	6111	\'à]	Ն÷ս 270	61 u	Les
	Pro	Phe	Նչ։ 275	63 y	Asp	Asp	lle	Th: 280	Met	Val	Leu	Ile	Leu 285	Pro	L ys	Pro
10	Glu	Lys 290	Ser	Leu	Αla	L;·s	Va1 295	Glu	Lys	Glu	Leu	Thr 300	Fro	Glu	Val	Let
	Gln 305	Glu	Trp	Leu	Asp	Glu 310	Leu	Сĵи	Glu	Met	Met 315	Leu	Vā]	Val	His	Met 320
15	Pro	Arg	Phe .	Arg	11e 325	Glu	Asp	ely	Phe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	Gln
	Asp	Met	Gly :	Leu 3 40	Va1	Asp	Leu	Phe	Ser 345	Pro	Glu	Lys	Ser	Lys 350	Leu	Pro
20		•	355					360					365		Ala	
		3 / 0					375				;	085				
25	Ser 3 385					390				-	395					400
	Thr F			4	205				4	410				•	415	
30	Leu A	sn T	hr I 4	le I 20	le F	he l	viet (krg \ 125	/al /	Ala 2	sn I		130 Sys /	Val I	bys
	(2) I	nfor	MATI:	ON F	or s	EQ I	0 N G): 4 2	·:							
? 5		(i	(A) (E)	LEN TYF	GTH: E: a	464 minc										
		ii) }				_										
0	Met Ty -32		er As				1у т	: SE(hr V: 25					ys A:	rg L	ys Va	a 1
5	Tyr Le	eu Le 15	υ Se	r Le	eu Le	∌u L -	eu 1. 10	le Gl	ly Pi	ne Ti	rp As -			el Tl	hr Cj	y 5
	His Gl l	y Se	r Pr	o Va	il As 5	p I.	le C	's Th		la 31 19	le Pr	o Ar	g Se		le F: 15	0
0	Met As	n Pr	o Me 2	τ C γ	s Il	е Ту	yr Ai	g Se 2	r Fr 5	'၁ G]	u Ly	s Ly		a Th 0	ır Gl	. น
-	Asp Gl	ນ Gl 3	չ, ջ. 2	r G]	u Gl	n L	's I) 4	e Pr	c Gl	u Al	aT a	r As		g As	g Va	1

Glu Lys Thr Ser Asp Gln Ile His Phe Phe Phe Ala Lys L 125 Arg Leu Tyr Arg Lys Ala Asn Lys Ser Ser Lys Leu Val S 130 Arg Leu Phe Gly Asp Lys Ser Leu Thr Phe Asn Glu Thr T 145 Ile Ser Glu Leu Val Tyr Gly Ala Lys Leu Gln Pro Leu A 166 Glu Asn Ala Glu Gln Ser Arg Ala Ala Ile Asn Lys Trp V 180 Lys Thr Glu Gly Arg Ile Thr Asp Val Ile Pro Ser Glu A 205 Glu Leu Thr Val Leu Val Leu Val Asn Thr Ile Tyr Phe L 210 Trp Lys Ser Lys Phe Ser Pro Glu Asn Thr Arg Lys Glu Leu 225 Lys Ala Asp Gly Glu Ser Cys Ser Ala Ser Met Met Tyr Glu Lys Phe Arg Tyr Arg Arg Val Ala Glu Gly Thr Gln Val Leu 245 Lys Phe Arg Tyr Arg Arg Val Ala Glu Gly Thr Gln Val Leu 265	Leu Ser Fr. 8 Ala Cys As: 95 Thr Ile Se. 110 Leu Asn Cy: Ser Ala Asr Tyr Gln Asr 160 Asp Phe Lys 175 /al Ser Asr
Leu Ser Ile Ser Thr Ala Phe Ala Met Thr Lys Leu Gly A 80 Asp Thr Leu Gln Gln Leu Met Glu Val Phe Lys Phe Asp T 100 Glu Lys Thr Ser Asp Gln Ile His Phe Phe Phe Ala Lys L 125 Arg Leu Tyr Arg Lys Ala Asn Lys Ser Ser Lys Leu Val S 130 Arg Leu Phe Gly Asp Lys Ser Leu Thr Phe Asn Glu Thr T 155 Arg Leu Phe Gly Asp Lys Ser Leu Thr Phe Asn Glu Thr T 155 Glu Asn Ala Glu Gln Ser Arg Ala Ala Ile Asn Lys Trp V 180 Lys Thr Glu Gly Arg Ile Thr Asp Val Ile Pro Ser Glu A 195 Glu Leu Thr Val Leu Val Leu Val Asn Thr Arg Lys Glu Leu 210 Trp Lys Ser Lys Phe Ser Pro Glu Asn Thr Arg Lys Glu Leu 225 Lys Ala Asp Gly Glu Ser Cys Ser Ala Ser Met Met Tyr Gl 260 Lys Phe Arg Tyr Arg Arg Val Ala Glu Gly Thr Gln Val Leu 220 Glu Lys Ser Leu Ala Lys Val Glu Lys Glu Leu Thr Pro Gl 290 Gln Glu Trp Leu Asp Glu Leu Glu Glu Met Met Leu Val Val 295 Gln Glu Trp Leu Asp Glu Leu Glu Glu Met Met Leu Val Val 305	Ala Cys Ass 95 Thr Ile Se. 110 Leu Asn Cys Ser Ala Asr Tyr Gln Asr 160 Asp Phe Lys 175 Val Ser Asr
Asp Thr Leu Gln Gln Leu Met Glu Val Phe Lys Phe Asp T 100	95 Thr Ile Se. 110 Leu Asn Cyr Ser Ala Asr Tyr Gln Asr 160 Asp Phe Lys 175 /al Ser Asr
Glu Lys Thr Ser Asp Gln Ile His Phe Phe Phe Ala Lys L 115	Leu Asn Cys Ser Ala Asn Tyr Gln Asn 160 Asp Phe Lys 175 Val Ser Asn
115 120 125 Arg Leu Tyr Arg Lys Ala Asn Lys Ser Ser Lys Leu Val S 130 145 145 120 145 155 140 177 155 146 145 120 145 125 146 145 125 126 140 145 125 126 140 145 125 126 140 145 125 126 146 125 126 140 145 125 126 140 145 125 126 140 145 125 126 140 145 126 126 126 126 126 126 126 126 126 126	Ser Ala Asr Tyr Gln Asr 160 Asp Phe Lys 175 Val Ser Asr
130 135 140 Arg Leu Phe Gly Asp Lys Ser Leu Thr Phe Asn Glu Thr T 155 Ile Ser Glu Leu Val Tyr Gly Ala Lys Leu Gln Pro Leu A 165 Glu Asn Ala Glu Gln Ser Arg Ala Ala Ile Asn Lys Trp Val 180 Lys Thr Glu Gly Arg Ile Thr Asp Val Ile Pro Ser Glu A 205 Glu Leu Thr Val Leu Val Leu Val Asn Thr Ile Tyr Phe Lyz 10 Trp Lys Ser Lys Phe Ser Pro Glu Asn Thr Arg Lys Glu Leu 230 Lys Ala Asp Gly Glu Ser Cys Ser Ala Ser Met Met Tyr Glu 245 Lys Phe Arg Tyr Arg Arg Val Ala Glu Gly Thr Gln Val Leu 260 Pro Phe Lys Gly Asp Asp Ile Thr Met Val Leu Ile Leu Pr 275 Glu Lys Ser Leu Ala Lys Val Glu Lys Glu Leu Thr Pro Glu 290 Gln Glu Trp Leu Asp Glu Leu Glu Glu Met Met Leu Val Val 305 Gln Glu Trp Leu Asp Glu Leu Glu Glu Met Met Leu Val Val 305	Tyr Gln Ass 160 Asp Phe Lys 175 Val Ser Ass
145 11e Ser Glu Leu Val Tyr Gly Ala Lys Leu Gln Pro Leu A 165 11e Ser Glu Leu Val Tyr Gly Ala Lys Leu Gln Pro Leu A 170 Glu Asn Ala Glu Gln Ser Arg Ala Ala Ile Asn Lys Trp V 180 Lys Thr Glu Gly Arg Ile Thr Asp Val Ile Pro Ser Glu A 205 Glu Leu Thr Val Leu Val Leu Val Asn Thr Ile Tyr Phe Ly 210 Trp Lys Ser Lys Phe Ser Pro Glu Asn Thr Arg Lys Glu Leu 235 Lys Ala Asp Gly Glu Ser Cys Ser Ala Ser Met Met Tyr Gl 245 Lys Phe Arg Tyr Arg Arg Val Ala Glu Gly Thr Gln Val Leu 260 Pro Phe Lys Gly Asp Asp Ile Thr Met Val Leu Ile Leu Pr 285 Glu Lys Ser Leu Ala Lys Val Glu Lys Glu Leu Thr Pro Gl 300 Gln Glu Trp Leu Asp Glu Leu Glu Glu Met Met Leu Val Va 305	160 Asp Phe Lys 175 Val Ser Asr
Glu Asn Ala Glu Gln Ser Arg Ala Ala Ile Asn Lys Trp V. Lys Thr Glu Gly Arg Ile Thr Asp Val Ile Pro Ser Glu A. 25 Glu Leu Thr Val Leu Val Leu Val Asn Thr Ile Tyr Phe L. 210 Trp Lys Ser Lys Phe Ser Pro Glu Asn Thr Arg Lys Glu Le 225 Lys Ala Asp Gly Glu Ser Cys Ser Ala Ser Met Met Tyr Gl 245 Pro Phe Lys Gly Asp Asp Ile Thr Met Val Leu Ile Leu Pr 286 Glu Lys Ser Leu Ala Lys Val Glu Lys Glu Leu Thr Pro Gl 290 Gln Glu Trp Leu Asp Glu Leu Glu Glu Met Met Leu Val Va 305	175 /al Ser Asn 190
Lys Thr Glu Gly Arg Ile Thr Asp Val Ile Pro Ser Glu A. 205 Glu Leu Thr Val Leu Val Leu Val Asn Thr Ile Tyr Phe L. 220 Trp Lys Ser Lys Phe Ser Pro Glu Asn Thr Arg Lys Glu Leu 235 Lys Ala Asp Gly Glu Ser Cys Ser Ala Ser Met Met Tyr Gl. 250 Lys Phe Arg Tyr Arg Arg Val Ala Glu Gly Thr Gln Val Leu 265 Pro Phe Lys Gly Asp Asp Ile Thr Met Val Leu Ile Leu Pr 285 Glu Lys Ser Leu Ala Lys Val Glu Lys Glu Leu Thr Pro Gl. 290 Gln Glu Trp Leu Asp Glu Leu Glu Glu Met Met Leu Val Va. 305	190
25 Glu Leu Thr Val Leu Val Leu Val Asn Thr Ile Tyr Phe Ly 210 Trp Lys Ser Lys Phe Ser Pro Glu Asn Thr Arg Lys Glu Le 225 Lys Ala Asp Gly Glu Ser Cys Ser Ala Ser Met Met Tyr Gl 245 Lys Phe Arg Tyr Arg Arg Val Ala Glu Gly Thr Gln Val Le 265 Pro Phe Lys Gly Asp Asp Ile Thr Met Val Leu Ile Leu Pr 285 Glu Lys Ser Leu Ala Lys Val Glu Lys Glu Leu Thr Pro Gl 290 Gln Glu Trp Leu Asp Glu Leu Glu Glu Met Met Leu Val Va 305	la Ile Asr
210 215 220 Trp Lys Ser Lys Phe Ser Pro Glu Asn Thr Arg Lys Glu Let 225 Lys Ala Asp Gly Glu Ser Cys Ser Ala Ser Met Met Tyr Gl 245 Lys Phe Arg Tyr Arg Arg Val Ala Glu Gly Thr Gln Val Let 265 Pro Phe Lys Gly Asp Asp Ile Thr Met Val Leu Ile Leu Pr 285 Glu Lys Ser Leu Ala Lys Val Glu Lys Glu Leu Thr Pro Gl 290 Gln Glu Trp Leu Asp Glu Leu Glu Glu Met Met Leu Val Val 305	
Lys Ala Asp Gly Glu Ser Cys Ser Ala Ser Met Met Tyr Gl 245 Lys Phe Arg Tyr Arg Arg Val Ala Glu Gly Thr Gln Val Le 260 Pro Phe Lys Gly Asp Asp Ile Thr Met Val Leu Ile Leu Pr 275 Glu Lys Ser Leu Ala Lys Val Glu Lys Glu Leu Thr Pro Gl 290 Gln Glu Trp Leu Asp Glu Leu Glu Glu Met Met Leu Val Va 305	ys Gly Leu
Lys Phe Arg Tyr Arg Arg Val Ala Glu Gly Thr Gln Val Le 260 Pro Phe Lys Gly Asp Asp Ile Thr Met Val Leu Ile Leu Pr 285 Glu Lys Ser Leu Ala Lys Val Glu Lys Glu Leu Thr Pro Gl 290 Gln Glu Trp Leu Asp Glu Leu Glu Glu Met Met Leu Val Va 305	eu Phe Tyr 240
Pro Phe Lys Gly Asp Asp Ile Thr Met Val Leu Ile Leu Pr 275 Glu Lys Ser Leu Ala Lys Val Glu Lys Glu Leu Thr Pro Gl 290 Gln Glu Trp Leu Asp Glu Leu Glu Glu Met Met Leu Val Va 305	ln Glu Gly 255
Glu Lys Ser Leu Ala Lys Val Glu Lys Glu Leu Thr Pro Gl 290 295 300 Gln Glu Trp Leu Asp Glu Leu Glu Glu Met Met Leu Val Va 305 310 315	eu Glu Leu 70
Gln Glu Trp Leu Asp Glu Leu Glu Glu Met Met Leu Val Va 305 310 315	ro Lys Pro
Gln Glu Trp Leu Asp Glu Leu Glu Glu Met Met Leu Val Va 305 310 315	lu Val Leu
Pro Arg Phe Arg Ile Glu Asp Gly Phe Ser Leu Los Glu Gl	al His Met 320
325 330	ln Leu Gln
Asp Met Gly Leu Val Asp Leu Phe Ser Pro Glu Lys Ser Ly 340 345 35	335
Gly Ile Val Ala Glu Gly Arg Asp Asp Leu Tyr Val Ser As 355 360 365	ys Leu Pro
50 His Lys Ala Phe Leu Glu Val Asn Glu Glu Gly Ser Glu Ala 370 375 380	ys Leu Pro 50

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forms the $\omega_{\rm tot}$, decomposition (

	38	5	I AJ	a va	ej Va	35 35		:u 6.	1y A	rg S		eu A. 95	sn P	rc As	en Ai	rg Val 400
5	ńŢ	r Ph	e Ly	's Al	a As 40		g P1	(o P)	ne L		10 10	ie 13	le A	rg Gi	iu Va 41	3) Pro
	Le	u As	n Th	= 11 42	e Il O	e Ph	e 1-1e	t GI		rg Va 25	נג ני	e As	in Pi	:0 C) 43		l Lys
10	(2) IN	гори	AT I O	07 M	R SE	Q ID	NO:	43:							
15				(A) (B)	TYPE Lype	E CH TH: : am LOGY	4.64 ino	amin acid	oa c							
		(i:	i) M	OLEC	ULE .	TYPE	pr	otei	n							
20						DESC										
20	Met - 32	Туз	- 30	C A.SI	n Val	! Ile	: Gl	- 2		1 Th	r Sei	r Gl	y Ly.		g Ly.	s Val
	Tyr	Le:	let b	Sei	. Le	ı Lev	-10	1 11 (e Gl	y Ph	e Trp	Asj -:		s Val	l Th	r Cys
25	His 1	e)	' Ser	Pro	Va]	Asp	ije	e Cys	5 Th	r Ala 10		Pro) Ar	j Asp	11e	e Pro
	Met	Asn	Pro	Met 20	Cys	Ile	Tyr	Arç	Se 1 25		o 61 u	Lys	Lys	Ala 30		: Glu
30	Asp	Glu	G1 y 35	Ser	Glu	Gln	L)'5	11e		o Glu	Ala	Thr	Asr 45		Arç	, Val
•	Тгр	Gl ນ 50	Leu	Ser	Lys	Ala	Asn 55	Ser	Arg	Phe	: Ala	Th: 60		Phe	Tyr	Gln
35	His 65	Leu	Ala	Asp	Ser	Lys 70	Asn	Ъsр	Asn	Asp	Asn 75		Phe	Leu	Ser	Pro 09
	Leu	Ser	Ile	Ser	Thr 85	Ala	Phe	Ala	Met	Thr 90		Leu	Gly	Ala	Cys 95	Asn
40	Asp	Thr	Leu	Gln 100	Gln	Leu	Met	Glu	V≥1 105	Phe	Lys	Phe	Asp	Thr 110	Ile	Ser
	Glu	Lys	Thr 115	Ser	Asp	Gln	Ile	His 120	Phe	Phe	Phe	Ala	Gln 125	Leu	Asn	Сле
⁴⁵ .	Arg	Leu 130	Туг	Gln	Asn	Als	Asn 135	Lys	Ser	Ser	Lys	Leu 140	٧a]	5er	Ala	Asn
	Arg 145	Leu	Phe	Gly	Asp	Lys 150	Ser	Leu	Thr	Phe	Asn 155	Glu	Thr	Tyr	Gln	Asp 160
50	Ile	Ser	Glu	Leu	Val 165	Tyr	G1 y	Ala	Lys	Leu 170	Gln	Pro	Leu	Asp	Phe 175	F7.2

		_														
	511	ı Asn	Ala	61 u 180	Gln	Ser	Λrg	Ala	Ala 185		: Asn	Lys	Trp	Va] 190		. Asn
5	Lys	The	Glu 195	Gly	Arg	Ile	Thr	Asp 200	Val	Ile	e Pro	Ser	G1 t 205		Ile	e Asn
	Glı	Leu 210	Thr	Val	Leu	Val	Leu 215	Val	Asn	Thr	lle	Tyr 220		Lys	(G1 s	' Leu
10	Trp 225	Lys	Ser	Lys	Phe	Ser 230	Pro	G1 u	Asn	Thr	Arg 235		Glu	Leu	Phe	Tyr 240
	Lys	Ala	Asp	Gly	Glu 245	Ser	Cys	Ser	Ala	Ser 250		Met	туг	Gln	Glu 255	
15	Lys	Phe	Arg	Tyr 260	Arg	Arg	Val	Ala	Glu 265	Gly	Thr	Gln	Val	Leu 270	Glu	
	Pro	Phe	Lys 275	Gly	Asp	Asp	Ile	Thr 280		Val	Leu	Ile	Leu 285			Pro
20	Glu	Lys 290	Ser	Leu	Ala	Lys	Val 295		Lys	Glu	Leu	Thr 300		Glu	Val	Leu
_	Gln 305	Glu	qıT	Leu	Asp	Glu 310	Leu	Glu	Glu	Met	Met 315	Leu	Val	Val	His	Met 320
25	Pro	Arg	Phe	Arg	11e 325	Glu	Asp	Gly	Phe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	Gln
20	Asp	Met	Gly	Leu 340	Val	Asp	Leu	?he	Ser 345	Pro	Glu	Lys	Ser	Lys 350	Leu	Pro
	Gly	Ile	Val 355	Ala	Gl u	Gly	Arg	Asp 360	Asp	Leu	Tyr	Val	Ser 365	Asp	Ala	Phe
30	His	Lys 370	Ala	Phe	Leu	Glu	Val 375	Asn	Glu	Glu		Ser 38G	G1 u	Ala	Ala	Ala
	Ser 385	Thr .	Ala '	Val	Val	Ile 390	Phe	Pro	Arg	Ser	Leu . 395	Asn	Pro	Asn	Arg	Val 400
<i>3</i> 5	Thr	Phe :	Lys /	Ala .	Asn 405	Arg	Pro	Phe		Val 410	Phe :	lle .	Arg		Val 415	Pro
	Leu	Asn :	Thr 1	le :	Ile .	Phe 1	Met (Gly	Arg '	Val .	Ala i	Asn !		Cys 430	Val	Lys
40																
	(2)	INFOR														
45		(2	(A) (B)	LEN TY	GTH:	464 mino	ACTER I ami D aci	ino a id		5						
		(ii)	MOLE	CULE	TYF	E: p	rote	in								
50		(xi)									•					
	Met 1 -32	yr S -	er A. 30	sn V	al I	le G	ly T	hr 7 25	al T	hr s	er G		ys <i>F</i> 20	Arg I	ys \	al

	τy	r Le:		Se:	r Leu) Let	1 Lei -10		e Gl	y Př	ie Tr	p A5		s Va) Th	r Cys
		s Gly	y Set	Pro	val S		1)6	÷ C;:	s Th		a Ly O	s Pr	s Ar	g As) P I J	e Fro 5
5)4e i	t Asr	n Pro	Met 20		Il e	туг	: Ar		r Pr 5	o 61	ს ბ უ:	s Ly:	s Al. 30	-	r Glu
10	Asp	6lı	2 Gly 35		Glu	Gln	Lys	: 11e	-	ა G]	u 2.2.	t Thi	A5:	. '	g Ar	g Vēl
70	Trp	61 u 50		Ser	Lys	Ala	Asn 55		. Ar	g Ph	e 141 a	± Th: 60		: Phe	= Ty.	r Gln
15	Hi = 65) Ala	Asp	Ser	Lys 70	A.s n	Asp	Ası	n As	p A.s.i 75		Phe	: Lei	se:	r Pro 80
15	Leu	Ser	Ile	Ser	Thr 65	Ala	Phe	Ala	Met	t Th.		. Leu	G] ?	· Ala	6 Cyr. 95	s Asn
20				100					105	5				110	;	Ser
20			115					120					125			Cy's
		130					135					140				Asn
25	145					150					155					Asp 160
					165					170	•				175	
				160					185			ГÀг	_	190		
			195					200				Ser	205			
35		210					215					Tyr 220			_	
	225			•	:	230					235	Lys				240
40					245					250		Met			255	-
				260					265			Gln		270		
45			275				:	280				Ile Thr	285		_	
		290				2	95					300				
50	Gln 305	J_U .	1 مر ـ .	J. U. J.		16	seu (- - 0 (ot n	. TE L	315	∍eu '	val '	val .	піѕ	Met 320

	Pr	o Ar	g Ph	e Ard	325		u As	o 61;	/ Phe	330		Ly:	s Glu	G]r	Leu 335	Gla
5	Asj	p Me	t Gl	y Lei 340	ı Va] O	As;	p Lei	ı 2h∈	Se: 345		o Glu	ı Lys	s Sei	1 Lys 350		Pro
	G1;	y :10	e Va:	l Ala 5	Glu	Gly	y Arq	360		Le.	ту:	(Va)	365		Ala	Phe
10	His	370	s Ala	a Phe	e Leu	Glu	u Va3 375		Glu	Glu	Gly	, Sei 380		ı Ala	Ala	Ala
	Se: 385	Thi	: Ala	a Val	. Val	Il∈ 390	e Val	. Pro	Arg	Ser	395		Pro) Asn	Arg	Val 400
15	Thi	Phe	Lys	s Ala	Asn 405) Pro	Phe	Leu	Va <u>l</u> 410		lle	Arg	, Glu	Val 415	Pro
	Lev	ı Asn	Thr	11e 420	Ile	Ph∈	. Met	Gly	Arg 425		Ala	Asn	Pro	430		Lys
20	(2)	INF	amso"	TION	FOR	SEQ	eI g	NO:	45:							
25		,	((SEQU A) L B) T	ENGT YPE: OPOL	H: 4 am.i OGY:	64 a no a lin	mino cid ear	aci							
				LECU QUEN			-			ID N	0:4	5:				
30	Met -32	Tyr	Ser ~30	Asn	Val	Ile	Gly	Thr -25	Val	Thr	Ser	Gly	Lys -20		Lys	Val
	Tyr	Leu -15	Leu	Ser	Leu	Leu	Leu -10	Ile	Gly	Phe	Trp	Asp -5	Суѕ	Val	Thr	Cys
35	His 1	Gly	Ser	Pro	Va 1 5	Asp	Ile	Cys	Thr	Ala 10	Lys	Pro	Arg	Asp	Ile 15	Pro
10	Met	Asn	Pro	Met 20	Cys	Ile	Туг	Arg	Ser 25	Pro	Glu	Lys	Lys	Ala 30	Thr	Glu
	Asp	Glu	G1 y 35	Ser	Glu	Gln	Lys	11e 40	Pro	Glu	Ala	Thr	45	Arg 	Arg	Val
15	Trp	Glu 50	Leu	Ser	Lys	Ala	Asn 55	Ser	Arg	Phe	Ala	Thr 60	Thr	Phe	Tyr	Gln
	His 65	Leu	Ala	Asp	Ser	Lys 70	Asn	Asp	Asn	Asp	Asn 75	Ile	Phe	Leu	Ser	Pro 08
o				Ser	85					90					95	
	Asp	Thr	Leu	Gln 100	Gln	Leu	Met		Val 105	Phe	Lys	Phe	Asp	Thr 110	lle	Ser

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Edistry Street, and the market for the

	63 1	n FA:	111		: As ₁	p 63	n Il	= Hi 32		e Fh	e Ph	e A <u>l</u>	a Gl 13		u As.	rı Cys
s	Ar	g Lev 130		Glr	, Ası	n 42	a As: 133		s Se	: Se	r Ly	5 Le) Se	r A).	a Asn
	Arq]45) Phe	617	' Asp	5 Ly.		Lei	u Th	r Pin	e Ası 159		ያ ፖስ.	r Ty.	r G);	n Asp 160
10	Ile	se:	G) u	Leu	Val 165		r Gly	, A.1:	ب.رج ج	: Lec 170) Pro	> Let) Ası	2 Phe 175	t Lys
	Glu) Asn	Ala	189 190		Se I	Arg	Ala	Ala 185		e Asn	. Lys	Tr	> Val		Asn
15	Lys	Thr	Glu 195		Arg	: Ile	+ Thr	Asp 200		llie	÷ Pro	Sē <u>r</u>	Glu 205		Ile	e Asn
	G) u	Ն շ ս 210		Val	Leu	Va]	Leu 215		. Asn	The	: Ile	Tyr 220		: Lys	: G17	. Len
20	Trp 225		Ser	Lys	Phe	Ser 230		Glu	Asn	The	Arg 235		G) u	Leu	Phe	Tyr 240
20	Lys	Αla	Asp	ej y	Glu 245		Суs	Ser	Ala	Ser 250		Met	Tyr	Gln	Glu 255	G1 y
0 5	Lys	Phe	Arg	Туг 260	Arg	Arg	Val	Ala	G1 u 265		Thir	Gln	Val	Leu 270		Leu
25	Pro	Phe	Lys 275	Сĵλ	Asp	Asp	Ile	Th: 280	Met	Val	Leu	11e	Leu 285		Lys	P.FO
	Glu	Lys 290	Ser	Leu . ·	<i>1</i> .1 a	Lys	Val 295	Glu	Lys	Ġ1 'n	Leu	Thr 300	?ro	Glu	Vai	Leu
30	Gln 305	Glu	Trp	Ŀευ	Asp	Glu 310	Leu	Glu	Glu	Met	Met 315	Leu	Val	Vāl	His	Met 320
	Pro	Arg	Phe	Arg	11e 325	Glu	Asp	63 y	Phe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	Gln
35	Asp	Met	Gly	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	Glu	Lys	Ser	Lys 350	Leu	Pro
	Gly		Val 355	Ala	Gl บ	Gly	Arg	Asp 360	Asp	Leu	Tyr	Val	5er 365	Asp	Ala	Phe
40	His	Lys 370	Ala	Phe	Leu	Glu	Val 375	Asn	G] ນ	Glu	Gly	Ser 380	G1 u	Ala	Ala	Ala
	Ser 385	Thr	Ala	Val	Val	Ala 390	Leu	Gly	Arg	Ser	Leu 395	Asn	Pro	A.sn	Arg	Val 400
45	Thr	Phe	Lys		Asn 405	Arg	Pro	Phe		Val 410	Phe	11e	Ærg	Glu	Val 415	Pro
	Leu	Asn	Thr	11e : 420	lle	Phe	Met		Arg 425	Val	Ala.	A.s n		Су5 430	Val	Ly's
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(2) INFORMATION FOR SEQ ID NO: 46:

			(i)	(A) (B)	UENC LENG TYPE TOPO	ΤΗ: : aπ	464 iino	amin acid	o ac	S: ids						
5		(i	i) M	OLEC	ULE	TYPE	: pr	otei	ח							
		(x	i) S	EQUE	NCE	DESC	FIPT	: NO I	SEQ	ID	NO:	46:				
10	Me -3:	t Ту 2	r Se -3	r As O	n Va	1 11	e Gl	y Th -2	r Va 5	l Th	r Se	r GJ	y Ly -2		g Ly	s Val
	Ty:	r Le	u Le 5	u Se	r Le	u Le	u Le -1	u Il O	e Gl	y Pho	e Tr	As ₁		s Va	l Th	c Cys
15	•	L				5				10)				1	5
				2					2	5				30)	
20	Asp	Glu	و G1 35	y Se	r Glu	ı G11	n Ly:	5 Ile 40	Pro	o Glu	Ala	Th	Asr 45		Arq	y Val
	Trp	5 Glv	ı Let	ı Se:	r Lys	s Ala	3 Ası 5	n Sei	Arq	g Phe	: Ala	Thi 60		Phe	туі	Gln
25	His 65	Lev	ı Ala	a Asp	o Ser	7 Lys	Ası)	n Asp) Asr	n Asp	Asr 75		Phe	Let	S€1	Pro 80
					r Thr 85	•				90					95	•
30				100					105	•				110	•	
			113		: Asp			120					125			
35 ·		130			Asn		135					140				
	145				Asp	150			•		155					160
40					Val 165					170					175	_
				180	Gln				185					190		
45			193		Arg			200					205			
		210			Leu		215					220				
50	223				Phe	230					235					240
50	Lys	Ala	Asp	G1 y	Glu 245	Ser	Cys	Ser	Ala	Ser 250	Met	Met	Tyr	Gln	Glu 255	Gly

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	Ly	s Phe	Arg	Tyr 260		Arg	(Va)	A.) a	61. 265		Thr	G) r	Va)	270		. Ն ես
5	Pr	o Phe	Lys 275	G) Y	Asp	Asp	lle	?hr 280		Val	Leu	Ιlε	Leu 285		ЬУS	Pro
	Gl	u Lys 290	Ser	Leu	Ala	Lys	Val 295		Lys	Glu	Lev	Th.r 300		ejn	۷æ۱	Leu
10	Gl: 305	n Glu S	Trp	Leu	Asp	Glu 310		Glu	61 u	Met	Met 315	Leu	Va)	Vė]	Eis	Met 320
	Pro	Arg	Phe	Arg	11e 325	Glu	Asp	G1 y	Phe	Ser 330	Leu	Lys	Glu	GJ ti	Leu 335	
15	Asp) Met	61 A	Leu 340	Vā]	Жsр	Leu	Phe	Ser 345	Pro	G1 u	Lys	Ser	Lys 350	Leu	Pro
		lle	355					360					365			
20		Lys 370					375		•			380				
	385					390					395					400
25		Phe		•	405					410.					415	
	Leu	Asr.	Thr 1	lle 1 120	[]e	Phe :	Met		Arg 425	Val.	Ala.	Asn .		Cys 430	Val	Lys
30	(2)	INFO	PMAT I	ON F	OR :	SEQ :	ID N	O: 4	7:							
<i>3</i> 5			(B)	LEN TYP TOP	GTH: PE: 2 POLOG	: 46 emino SY: 1	4 am o ac line	ino d id ar	ICS: acid	s						
		(ii) (xi)							EQ 11	о ио:	47:			•		
40	. Met -32	Tyr S	er A 30	sn V	al I	le G		Thr \ -25	/al 7	Thr S	er G		ys A 20	Arg I	r), e v	/al
	Туг	Leu 1 -15	eu S	er L	eu L	eu l -	eu 1	le G	Sly F	he T	rp A	sp C -5	ys \	/al J	hr C	:ys
45	His 1	Gly S	er P.	ro V	al A 5	sp J] ∈ C	ys T	hr A	.la L 10	ys P	ro A	rg Æ	sp I	le F 15	ro
50	Met .	Asn P	ro Me	et Cy 20	ys I	le T	yr A		er P 25	rc G	lu L	ys L		la T 30	hr G	31 u
50	Asp (Glu G	ly 56 35	er Gl	lu G.	ln L	ys I	le P 40	ro G	lu'A	la Ti		sn A 45	rg A	rg V	al

	T	fp G1 5	u Le O	u Se	r Ly	s Al	a As 5	n Se S	r Ar	g Ph	e Al	a Th 6		r Ph	е Ту	r Gln
5	Hi E	s Le	u Ala	a As	p Se	r Ly 7	s As O	n As	p As	n Asj	o Asi	n Il 5	e Ph	e Le	u Se	r Pro 80
	Le	u Se	r Ile	e Se	Th.	r Al 5	a Ph	e Al	a Me	t Th:		s Le	u Gl	y A1	а Су 9	s A.sn S
10	As	p Th	r Lei	1 Glr 100	n Gl:	n Le	u Me	t Gl	u Va:	l Phe S	e Lys	s Ph	e As	P Th:		e Ser
	Gl	u Ly:	5 Th: 115	Ser	Asp	o Gla	n Ile	E His 120	s Phe	e Ph€	Phe	≥ Ala	a Gl:		ı Ası	n Cys
15		130)				135	•				140)			a Asn
	14.	5				150	י				155	•				1 Asp 160
20					165	•				170	'				175	
				180					185	•				190	•	: Asn
25			195					200	1				205	i		. Asn
		210					215					220				Leu
30	223					230	1				235					240
		Ala			245					250					255	_
3 5		Phe		260					265					270		
		Phe	215					280					285			
40		Lys 290					295					300				
	303	Glu				310					315					320
45		Arg			343					330					335	
		Met		340					345					350		
50			222					360					365			
	nıs	Lys 370	ATA	rhe :	Leu (Glu	Val . 375	Asn	Glu	Glu		Ser 380	Glu	Ala .	Ala	Ala

	Se 38		= A.1	ь Va) Va	1 13 39		1 -1	's Ar	g Se	er Le 39		in Pg	o As	n Ar	9 Va
5	T'n	r Ph	ē Ly	s 7.]	a A.S. 40		g Fr	o Pi	e Le	υ Va 43		e Il	e Ar	g G)	u Va 41	1 Pr 5
	Lei	u Ası	n Th	r 11 42		e Ph	e Me	t G1	y Ar 42		1 A1	a As	n Pr	o Cy 43		l Ly
10	(2)) IN	FOPM	ET10	N FOI	R SE	9 I D	но:	48:							
15				(A) I	JENCI LENGT LYPE : TOPOI	rH: 4	464 Lno	amin acid								
75		(11	L) MC	DLECU	ILE 1	TYPE:	pr:	orei:	n							
		(×i	.) 53	QUEN	ICE D	ESCF	RIPT	ION:	SEQ	ID	10: 4	£:				
20 .	Met -32		-30		Val	Ile	: G1	7 Th:		l Thi	r Se	: G1y	/ Lys		l Tys	s Val
	Tyr	-15	Leu	Ser	Leu	Leu	-10		: Gl	? Phe	a Trp	Asp -5		v≥l	Thi	Cys
25	His 1		Ser	Prc	Val 5		Ile	Cys	Thi	: Ala		Pro	Arg) Asp	11e	e Pro
	Met	Asn	Pro	Мет 20		Ile	Tyr	Arg	5er 25		o Glu	Lys	Lys	Ala 30		: Glu
30	Asp	Glu	G1 y 35		Glu	Gĺn	Lуs	11e		Glu	ı Ala	Thr	Asn 45		Arg	Val
	Trp	Glu 50		Ser	Lys	Ala	Asn 55		Arg	Phe	1.la	Thr 60		Phe	туг	Gln
35	His 65		Ala	Asp	Ser	Lys 70		Asp	Asn	Asp	Asn 75	Ile	Phe	Leu	Ser	Pro 08
	Leu	Ser	Ile	Ser	Thr 85	Ala	Phe	Ala	Met	Thr 90		Leu	Gl y	Ala	Cys 95	Asn
10	Asp	Thr	Leu	Gl n 100	Gln	Leu	Met	Glu	Val 105	Phe	Lys	Phe	Asp	Thr 110	Ile	Ser
•	Glu	Lys	Thr 115	Ser	Asp	Gln	Ile	His 120	Phe	Phe	Phe	Ala	Gln 125	Leu	Asn	Cys
15	Gln	Leu 130	Tyr	Gln	Asn	Ala	Asn 135	Lys	Ser	Ser	Ly.s	Leu 140	Val	Ser	A.1 a	Asn
	Arg 145	Leu	Phe	e1 y	Asp	Lys 150	Ser	Leu	Thr	Phe	Asn 155	Glu	Thr	Tyr	Gln	Asp 160
o	Ile	Ser	Glu	Leu	Val 165	Tyr	G1 y	Ala	Lys	Leu 170	Gla	Pro	Leu	Asp	Phe 175	Lys
v	Glu	Asn	.la	Glu 180	Gln	Ser	Arg	Ala	Ala 185	Ile	Asn	s'زل	Trp	Vel 190	Ser	Asn

	Ly	s Thi	r Glu	ر G1 د	, Arg	ııla	⊋ ፖክ:	: Ass	r Va.	1 116	e Pro	o Sei	c Gli	בוֹא נ		e Asn
			19.	,				200)				209	ò		
5		211	,				21:	,				220)			; Leu
	T:p 225	Lys S	Ser	Lys	Phe	Ser 230	Pro	: Glu) Asr	Thr	235	PAs	Glu	: Leu	Phe	Ty: 240
10	Lys	: Ala	Asp	Gly	Glu 245	Ser	Cys	Ser	Ala	Ser 250	Met	Met	Туг	: Gln	Glu 255	Gly
	Lys	Phe	Arg	Tyr 260	Arg	Arg	Val	Ala	Glu 265	Gly	Thr	Gln	Val	Leu 270		Leu
15	Pro	Phe	Lys 275	Gly	Asp	Asp	Ile	Thr 250	Met	Val	Leu	Ile	Leu 285		Lys	Pro
	Glu	Lys 290	Ser	Leu	Ala	Lys	Val 295	Glu	Lys	Glu	Leu	Thr 300		Glu	Val	Leu
20	Gln 305	Glu	Trp	Leu	Asp	Glu 310	Leu	Glu	Glu	Met	Met 315	Leu	Val	Val	His	Met 320
25	Pro	Arg	Phe	Arg	11e 325	Glu	Asp	Gly	Phe	Ser 330	Leu	Lys	Glu	Gln	Leu 335	
	Asp	Met	Gly	Leu 340	Val	Asp	Leu	Phe	Ser 345	Pro	Glu	Lys	Ser	Lys 350	Leu	Pro
30	Gly	Ile	Val 355	Ala	Glu	Gl y	Arg	Asp 360	Asp	Leu	Tyr	Val	Ser 365	Asp	Ala	Phe
	His	Lys 370	Ala	Phe	Leu	Glu	Val 375	Asn	Glu	Glu	Gly	Ser 380	Glu	Ala	Ala	Ala
35	Ser 385	Thr	Ala	Val	Val	Ala 390	Leu	Gl y	Arg	Ser	Leu 395	Asn	Pro	Asn	Arg	Val 400
	Thr	Phe	Lys	Ala	Asn . 405	Arg	Pro	Phe	Leu	Val 410	Phe	Ile	Arg	Glu	Val 415	Pro
40	Leu	Asn	Thr	Ile 420	Ile	Phe	Met	Gly.	Arg 425	Val	Ala	Asn	Pro	Cys 430	Val	Lys

	(2) INFORMATION FOR SEQ ID NO: 49:	
ွ 5	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 24 base pairs (B) TYFE: nucleic acid (C) STFANDEDNESS: single (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
10		
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:	
	GTTTAGCGAC CGCGGAGCAA TCAC	2
15	(2) INFORMATION FOR SEQ ID NO: 50:	
	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 33 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
20	(ii) MOLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:	
25	GGGGTTTAGC GACCGCGGAA AAATCACAAC AGC	33
	(2) INFORMATION FOR SEQ ID NO: 51:	
30	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 30 base pairs (E) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
35		
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:	
	TAGCGAACGG CCGACAGCCA CAACAGCGGT	30
40	(2) INFORMATION FOR SEQ ID NO: 52:	
45	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 22 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
50	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:	
	CAGCGGTACT GCCAGCTGCT TC	22

	(2) INFORMATION FOR SEQ ID NO: 53:	
5	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 27 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
10		
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:	
	ACGGCCAGCA ATCGGAACAG CGGTACT	27
15	(2) INFORMATION FOR SEQ ID NO: 54:	
20	 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 23 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear 	
	(ii) MOLECULE TYPE: DNA (genomic)	
25	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:	
	AATCACAACA AAGGTACTTG CAG	23
	(2) INFORMATION FOR SEQ ID NO: 55:	
30	 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 30 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear 	
35	(ii) MOLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:	
40	GTTTAGCGAA CGCGGAATAA TCACAACAGC	30
	(2) INFORMATION FOR SEQ ID NO: 56:	
45	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 29 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
50	(vi) SEQUENCE DESCRIPTION	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:	

55

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	GTTTAGCGAA CGCGGACCAA TCADAACAC	25
	(2) INFORMATION FOR SEC 15 NO: 57:	
5	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 30 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
10	(ii) MOLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:	
15	GTTTAGCGAA CGCGGATAAA TCACAACAGC	30
	(2) INFORMATION FOR SEQ ID NO: 56:	
20	(i) SEQUENCE CHAFACTERISTICS: (A) LENGTH: 30 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
25		
23	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:	
	GTTTAGCGAA CGCGGCCAAA TCACAACAGC	30
30	(2) INFORMATION FOR SEQ ID NO: 59:	
30	 (i) SEQUENCE CHAPACTERISTICS: (A) LENGTH: 29 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear 	
3 5	(ii) MGLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:	
40	GTTTAGCGAA CGCGGAACAA TCACAACAG	29
	(2) INFORMATION FOR SEQ 1D NO: 60:	
45	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 30 base pairs (E) TYPE: nucleic acid (C) STRANDEDHESS: single (D) TOPCLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
50		
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:	

115

	TAGCGAACGG CCAATAGCCA CAACAGCGGT	30
	(2) INFORMATION FOR SEQ ID NO: 61:	
5	 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 30 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear 	
10	(ii) MOLECULE TYPE: DNA (genomic)	
•=	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:	
15	TAGCGAACGG CCAAGAGCCA' CAACAGCGGT	30
	(2) INFORMATION FOR SEQ ID NO: 62:	
20	 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 29 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear 	
25	(ii) MOLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 62:	
30	TAGCGAACGG CCAAGACCCA CAACAGCGG	29
30	(2) INFORMATION FOR SEQ ID NO: 63:	
35	 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 34 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear 	
	(ii) MOLECULE TYPE: DNA (genomic)	
40		
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 63:	
	GTTTAGCGAA CGGGGAACAG CCACAACAGC GGTA	34
45	(2) INFORMATION FOR SEQ ID NO: 64:	
5 <i>0</i>	 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 34 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear 	
	(ii) MOLECULE TYPE: DNA (genomic)	

	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 64:	
	GTTTAGCGAA CGGGGAAAAA GCACAACAGC GGTA	34
5	(2) INFORMATION FOR SEQ 1D NC: 65:	
10	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 30 base pairs (B) TYPE: nucleic acid (C) STRAMDEDNESS: single (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
15	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 65:	
	GTTTAGCGAA CGCGGAAGAA TCACAACAGC	30
	(2) INFORMATION FOR SEQ ID NO: 66:	
20	(i) SEQUENCE CHAPACTERISTICS: (A) LENGTH: 34 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
25	(ii) MOLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 66:	
30	GTTTAGCGAA CGCGGATAAG CCACAACAGC GGTA	34
	(2) INFORMATION FOR SEQ ID NO: 67:	
35	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 33 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
40		
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 67:	
	GTTTAGCGAA CGCGGCCAAG CCACAACAGC GGT	33
45	(2) INFORMATION FOR SEQ ID NO: 68:	
50	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 33 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	

	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 68:	
	GTTTAGCGAA CGCGGCCAAA GCACAACCGA GGT	33
5	(2) INFORMATION FOR SEQ ID NO: 69:	
10	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 29 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
	(ii) MCLECULE TYPE: DNA (genomic)	
15	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 69:	
	GAAAGTCACC CTCTCGGGGT TTAGCGAAC	29
	(2) INFORMATION FOR SEQ ID NO: 70:	
20	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 32 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
25	(ii) MOLECULE TYPE: DMA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 70:	
3 0	TTGAAAGTCA CCCTCCTCGG GTTTAGCGAA CG	32
	(2) INFORMATION FOR SEQ ID NO: 71:	
35	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 32 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
40		
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 71:	
	TTGAAAGTCA CCCGTCGACG GTTTAGCGAA CG	32
45	(2) INFORMATION FOR SEQ ID NO: 72:	
50	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 27 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
= #	(ii) MOLECULE TYPE: DNA (genomic)	

	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 72:	
	CGGCAGTTCA GTTGGGCAAA GAAGAAG	27
s	(2) INFORMATION FOR SEQ ID NO: 73:	
10	(i) SEQUENCE CHAPACTERISTICS: (A) LENGTH: 30 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic) .	
75	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 73:	
	GGATTTGTTG GCGTTTTGAT AGAGTCGGCA	30
20	(2) INFORMATION FOR SEQ ID NO: 74: (i) SEQUENCE CHAPACTERISTICS:	
	(A) LENGTH: 19 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
25	(ii) MOLECULE TYPE: DNA (genomic)	
.30	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 74:	
	GATAGACTTG GCAGTTCAG	19
	(2) INFORMATION FOR SEQ ID NO: 75:	
35	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 21 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
40	(ii) MOLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 75:	
4 5.	GGTGGCCTCC AGGATCTTCT G	21
	(2) INFORMATION FOR SEQ ID NO: 76:	
50	(i) SEQUENCE CHAPACTERISTICS: (A) LENGTH: 39 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	

(ii) MOLECULE TYPE: DNA (genomic)

5	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 76:	
	GGGATTCATG GGAATGGATC GTGGGATTGC TGTGCAGAT	39
	(2) INFORMATION FOR SEQ ID NO: 77:	
10	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 21 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
15	(ii) MOLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 77:	
20	GTTGGCTTTT TGATAGAGTC G	21
	(2) INFORMATION FOR SEQ ID NO: 78:	
25	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 21 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
30	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 78:	
•	TTTGTTGGCG TTTCGATAGA G	21
•	(2) INFORMATION FOR SEQ ID NO: 79:	
35	 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 21 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear 	
40	(ii) MOLECULE TYPE: DNA (genomic)	
	(x1) SEQUENCE DESCRIPTION: SEQ ID NO: 79:	
45	TTTGTTGGCT TGTCGATAGA G	21
	(2) INFORMATION FOR SEQ ID NO: 80:	
50	 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 25 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear 	

	(ii) MOLECULE TYPE: DNA (genomic)	
s	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 80:	
	TACATGGCCG AAGCTTCGTA ATCAT	25
10	(2) INFORMATION FOR SEQ ID NO: 81:	
	 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 29 base pairs (B) TYPE: nucleic acid (C) STPANDEDNESS: single (D) TOPOLOGY: linear 	
15	(ii) MOLECULE TYPE: DNA (genomic)	
20	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 81:	
	CANAGNATAN GATCTTATTA CTTANCACA	29
25	Claims	
30	1. A human antithrombin III (AT III) mutant obtained by subjecting human AT III to mutation, which human AT III amino acid sequence described below except that an amino acid(s) mutates into an amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41- to positions, the 125- to 133-positions and the 384- to 398-positions:	other
35		
10		
	·	
15		

50

human AT III amino acid sequence

5																	
					Asn	Va l	lle	Gly		Val	Thr	Ser	Gly	Lys	Arg	Lys	Val
		-32		-30					-25					-20			
10		Туг	Leu	Leu	Ser	Leu	Leu	Leu	ile	Gly	Phe	Trp	Asp	Cys	Va l	Thr	Cys
			-15					-10					-5				
		His	Gly	Ser	Pro	Va l	Asp	lle	Cys	Thr	Ala	Lys	Pro	Агд	Asp	Πe	Рго
15		1				5					10					15	
		Met	Asn	Pro	Met	Cys	lle	Tyr	Arg	Ser	Pro	Glu	Lys	Lys	Ala	Thr	Glu
					20					25					30		
20		Asp	Glu	Gly	Ser	Glu	Gln	Lys	He	Pro	Glu	Ala	Thr	Asn	Arg	Arg	Val
				35					40				·	45			
		Trp	Glu	Leu	Ser	Lys	Ala	Asn	Ser	Arg	Phe	Ala	Thr	Thr	Phe	Tyr	Gln
25			50					5 5					60				
		His	Leu	Ala	Asp	Ser	Lys	Λsn	Asp	Asn	Λsp	Asn	lle	Phe	Leu	Ser	Pro
	•	65					70					75					80
30		Leu	Ser	He	Ser	Thr	Ala	Phe	Ala	Met	Thr	Lys	Leu	Gly	Ala	Cys	۸sn
30						85					90					95	
35																	
40																	
45																	
50																	

	Asp	Thr Leu	Gln Gl	n Leu	Met	Glu Va	l Phe Ly	s Phe A	sp Thr	lle Ser
			100			10	5		110	
5	G1 u	Lys Thr	Ser As	p Gln	He	llis Pho	e Phe Ph	e Ala <u>L</u>	ys Leu	Asn Cys
		115	i		į	20		1	25	
	Arg	Leu Tyr	Arg Ly	<u>s</u> Ala	Asn l	Lys Sei	Ser Ly	s Leu Y	al Ser	Ala Asn
70		130			135			140		
	Arg	Leu Phe	Gly As	p Lys	Ser L	eu Thr	Phe As	n Glu Ti	hr Tyr	Gln Asp
	145			150			15	5		160
75	lle	Ser Glu	Leu Va	Tyr	Gly A	la Lys	Leu Gl	n Pro Le	qzA us	Phe Lys
			16				170			175
	Glu	Asn Ala		Ser	Arg A	la Ala	ile Ası	Lys Ti	p Val	Ser Asn
20			180			185			190	
	Lys		Gly Arg	lle			lle Pro	Ser Gl	u Ala	lle Asn
		195				00		20		•
25			Val Leu			al Asn	Thr ile	: Tyr Ph	e lys	Gly Leu
		210			215			220		
		Lys Ser	Lys Phe		Pro G	lu Asn	Thr Arg		u Leu	Phe Tyr
30	225			230			235			240
	Lys A	lla Asp			Cys Se	er Ala	Ser Met	Met Ty		
	1 D	16 - 4	245				250			255
	Lys P			Arg	rai Ai		Gly Thr	GIn Va		Glu Leu
35	9 D		260 Clu Ann		. 1 - TL	265	W-1 1		270	
	710 F	275	uly Asp	ASP			Val Leu			Lys Pro
	Clu l		iou Alo	lvo l	28		Glu Leu	285		U. 3. I
40		90	ren via		95	n ris	din Lea	300		Ast ren
			Leu Asn			n Glu	Met Met		Val I	Jic Not
	305	р		310		0 010	315	Leu vai	141 1	320
45		rg Phe <i>I</i>	Arg lle		sp G1	y Phe	Ser Leu	Lys Glo	Gln i	
		= ' '	325		, ,,		330	-,- 016		35
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Asp Met Gly Leu Val Asp Leu Phe Ser Pro Glu Lys Ser Lys Leu Pro Gly lle Val Ala Glu Gly Arg Asp Asp Leu Tyr Val Ser Asp Ala Phe His Lys Ala Phe Leu Glu Val Asn Glu Glu Gly Ser Glu Ala Ala Ala Ser Thr Ala Val Val Ile Ala Gly Arg Ser Leu Asn Pro Asn Arg Val Thr Phe Lys Ala Asn Arg Pro Phe Leu Val Phe Ile Arg Glu Val Pro Leu Asn Thr lle lle Phe Met Gly Arg Val Ala Asn Pro Cys Val Lys

District Control Committee : .

- The human AT III mutant as claimed in Claim 1, wherein said another amino acid(s) is selected from the group consisting of Ala, Gly, Trp, Pro, Leu, Val, Phe, Tyr, Ile, Glu, Ser, Gln, Asn and Arg.
- 3. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 384- to 398-positions and that an amino acid(s) mutates into another amino acid(s) at a region(s) selected from the group consisting of the 11-to 14-positions, the 41- to 47-positions and the 125- to 133-positions.
- 4. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 384- to 398-positions and that an amino acid(s) mutates into another amino acid(s) at a region(s) selected from the group consisting of the 11-to 14-positions and the 41- to 47-positions.
- 5. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 384- to 398-positions and that an amino acid(s) mutates into another amino acid(s) at a region(s) selected from the group consisting of the 11-to 14-positions and the 125- to 133-positions.
- 6. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 384- to 398-positions and that an amino acid(s) mutates into another amino acid(s) at a region(s) selected from the group consisting of the 41-to 47-positions and the 125- to 133-positions.
- 7. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 11- to 14-positions and that an amino acid(s) mutates into another amino acid(s) at the 384-to 398-positions.
- 50 8. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 41- to 47-positions and that an amino acid(s) mutates into another amino acid(s) at the 384-to 398-positions.
- 9. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 125- to 133-positions and that an amino acid(s) mutates into another amino acid(s) at the 384-to 398-positions.

- 10. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that an amino acid(s) mutates into another amino acid(s) at the 384- to 398-positions.
- 11. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that an amino acid(s) at the 384- to 398-positions mutates into another amino acid(s) selected from the group consisting of Ala, Pro, Leu, Val, Gly, Arg, Glu and Phe and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41-to 47-positions and the 125- to 133-positions.
- 12. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that an amino acid(s) at the 390- to 392-positions mutates into another amino acid(s) selected from the group consisting of Ala, Pro, Leu, Val and Phe and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41- to 47-positions and the 125- to 133-positions.
 - 13. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that Gly at the 392-position mutates into Pro and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41- to 47-positions and the 125- to 133-positions.
 - 14. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that a mutation selected from the group consisting of a mutation of IIe at the 390- position into AIa, a mutation of AIa at the 391- position into Phe, Val or Leu and a mutation of GIy at the 392-position into Pro is present and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41-to 47-positions and the 125- to 133-positions.
 - 15. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that a mutation selected from the group consisting of a mutation of Ala at the 384- position into Gly, a mutation of Ala at the 387- position into Phe, a mutation of Val at the 389-position into Pro, a mutation of Pro at the 397- position into Arg and a mutation of Asn at the 398-position into Glu or Alg is present and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41-to 47-positions and the 125- to 133-positions.
- 16. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that a mutation selected from the group consisting of a mutation of Lys at the 11- position into IIe, a mutation of Asp at the 14- position into Ser is present and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 41- to 47-positions, the 125-to 133-positions and the 384- to 398-positions.
 - 17. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that a mutation selected from the group consisting of a mutation of Lys at the 11- position into IIe and a mutation of Asp at the 14- position into Ser, and, another mutation selected from the group consisting of a mutation of IIe at the 390- position into Ala, a mutation of Ala at the 391- position into Phe, Val or Leu and a mutation of Gly at the 392-position into Pro are present, and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 41- to 47-positions and the 125- to 133-positions.
- 18. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that a mutation selected from the group consisting of a mutation of Lys at the 125- position into Gln, a mutation of Arg at the 129- position into Gln, a mutation of Lys at the 133- position into Asn or Gln is present and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 11- to 14-positions, the 41-to 47-positions and the 384- to 398-positions.
 - 19. The human AT III mulant as claimed in Claim 1, which has human AT III amino acid sequence except that a mulation selected from the group consisting of a mulation of Lys at the 125- position into Gln, a mulation of Arg at the 129- position into Gln, a mulation of Arg at the 132-position into Gln and a

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mutation of Lys at the 133- position into Asn or Gln, and, another mutation selected from the group consisting of a mutation of Ile at the 390- position into Ala, a mutation of Ala at the 391- position into Phe, Val or Leu and a mutation of Gly at the 392-position into Pro are present, and that an amino acid(s) may mutate into another amino acid(s) at a region(s) selected from the group consisting of the 11-to 14-positions and the 41- to 47-positions.

- 20. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that Gly at the 392-position mutates into Pro.
- 21. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that Ala-Gly at the 391- to 392-positions mutates into Phe-Pro.
 - 22. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that IIe-Ala at the 390- to 391-positions mutates into Ala-Leu.
 - 23. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that Lys at the 125-position mutates into Gln and Ala-Gly at the 391- to 392-positions mutates into Phe-Pro.
- 20 24. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that Arg-Lys at the 132- to 133-positions mutates into Gln-Asn and IIe-Ala at the 390- to 391-positions mutates into Ala-Leu.
- 25. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that Arg-Lys at the 132- to 133-positions mutates into Gln-Asn and Ala-Gly at the 391- to 392-positions mutates into Phe-Pro.
- 26. The human AT III mutant as claimed in Claim 1, which has human AT III amino acid sequence except that Lys at the 133-position mutates into Asn and Ala-Gly at the 391- to 392-positions mutates into Phe30
 Pro.
 - 27. A DNA coding for the human AT III mutant as claimed in Claim 1.
- 28. An expressible vector which has a DNA containing part or the whole of the DNA sequence coding for the human AT III mutant as claimed in Claim 1.
 - 29. A transformant which is obtained by subjecting host cells to transformation with the expressible vector as claimed in Claim 28.
- 40 30. The transformant as claimed in Claim 29, wherein the host cells are Escherichia coli or animal cells.
 - 31. A method for producing a human AT III mutant which comprises incubating the transformant as claimed in Claim 30 and recovering the human AT III mutant produced by the transformant from the culture.
- 32. A drug composition for thrombotic disorders which contains the human AT III mutant as claimed in Claim 1 and pharmaceutically acceptable carriers.
 - 33. A use of the human AT III mutant as claimed in Claim 1 for the making of a medicament for treating thrombotic disorders.

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Fig. 1

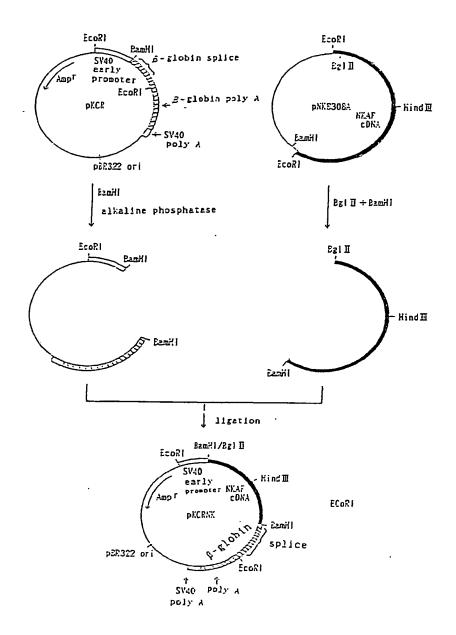


Fig. 2

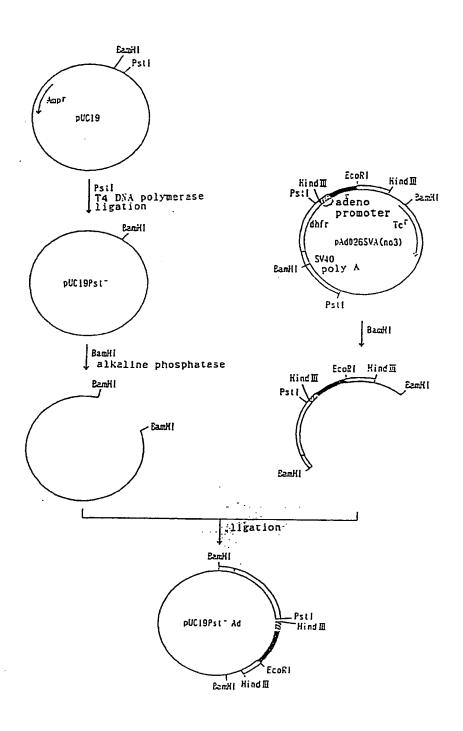


Fig. 3

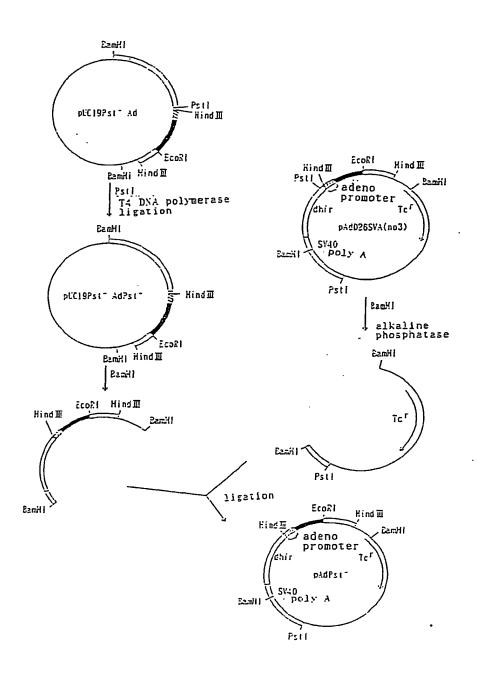


Fig. 4

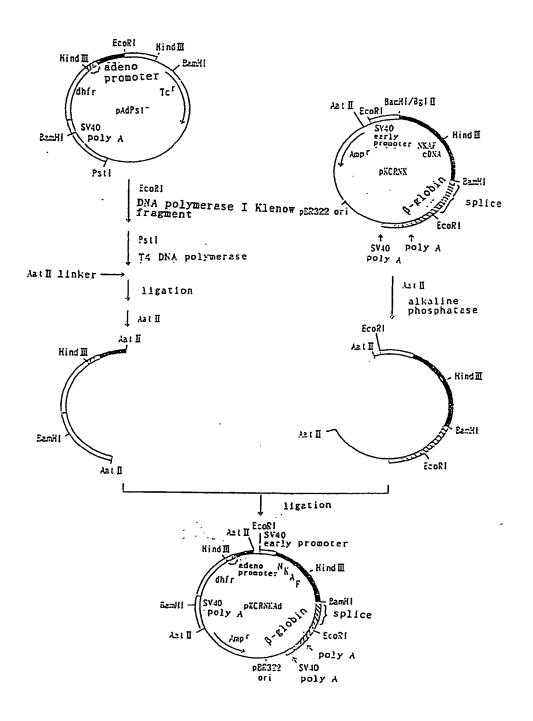


Fig. 5

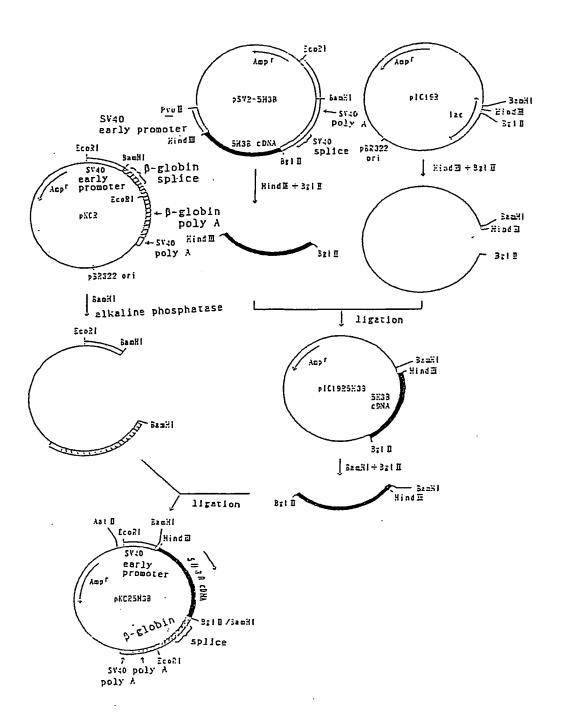


Fig. 6

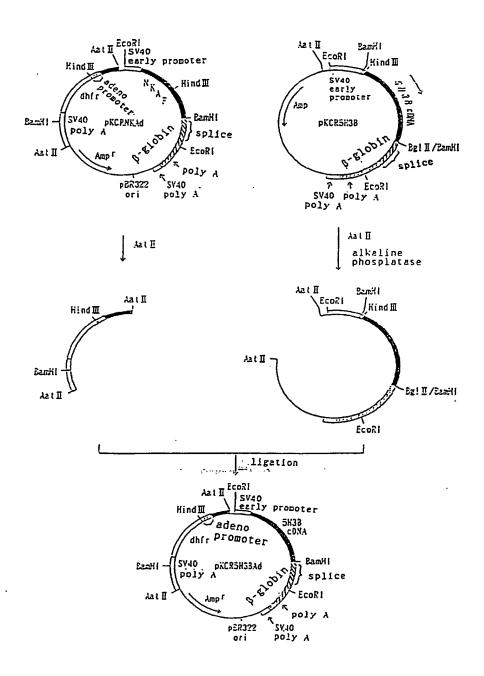
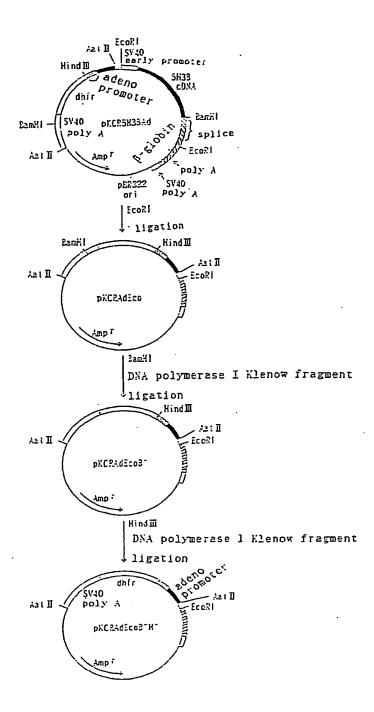


Fig. 7



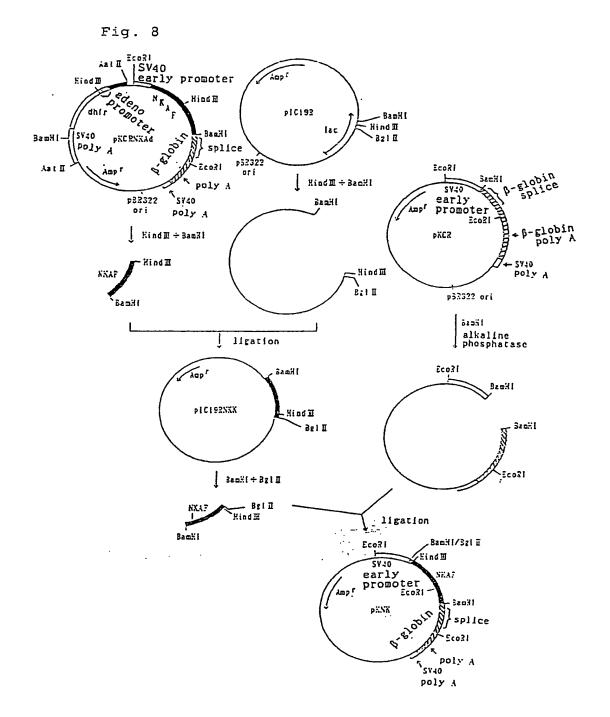


Fig. 9

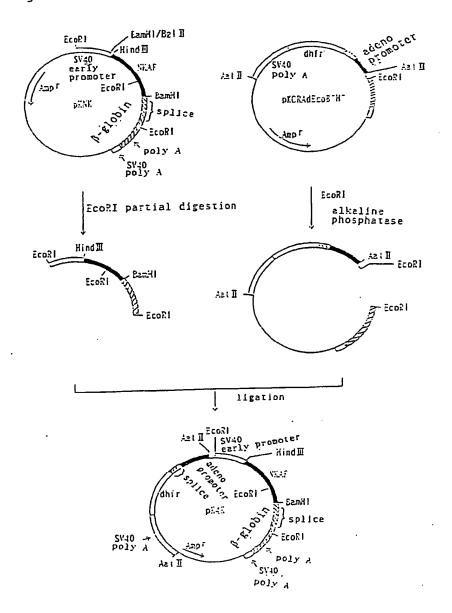
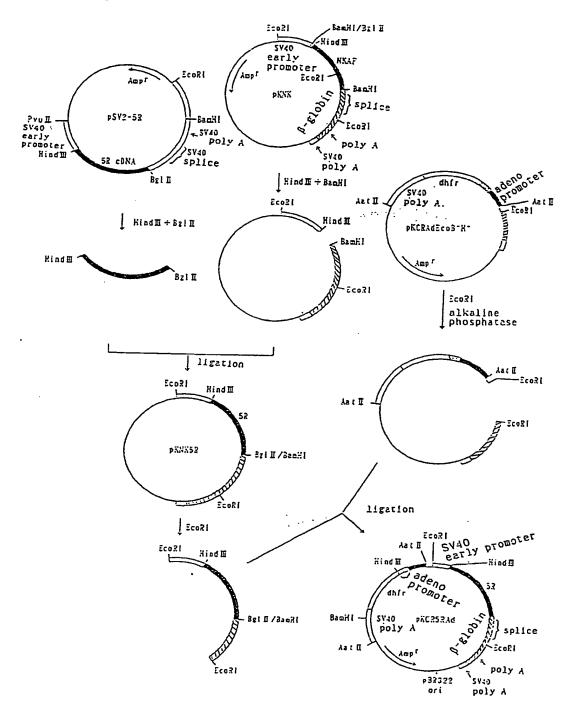


Fig. 10

First transfer of the second contract to ρ



EP 93 10 5829

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Caretor),	Citation of document with of relevant p	indication, where appropriate,	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Ct.5)				
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	* the whole docume	nt *	27-33					
\	EP-A-0 424 351 (WAS * abstract; claims	SHINGTON UNIVERSITY) 1-10 *	1,27-33					
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				TECHNICAL FIELDS SEARCHED (Int. Cl.5)				
				C07K				
				•				
	The present search report has t	een drawn up for all claims						
	Place of search	Date of completion of the search	<u></u>	Examiner				
В	ERLIN	12 JULY 1993 .		GURDJIAN D.				
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